The impact of APOE-ε4 status on sleep, rest-activity patterns and spatial navigation in healthy adults

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Abstract

INTRODUCTION

Alzheimer's disease(AD) is the most common type of dementia manifesting mainly over the age of 65 with no curative treatment available. The risk of AD is increased in APOE-ε4 allele carriers and those with sleep and circadian disturbances. APOE-ε4 polymorphism was also shown to be associated with spatial navigation impairment, which has been proposed to serve as a potential early marker of AD. Yet, the interrelationships between APOE-ε4 carriership, sleep, rest-activity patterns and spatial navigation in healthy older adults are still unclear. The presented PhD project addresses this research gap.

METHODS

One-hundred-sixty-one healthy participants took part in extensive screening sessions (51 APOE-ε4+, age($M \pm SD$)=63.18±7.84; 110 APOE-ε4-, age($M \pm SD$)=65.66±9.98) of which fifty-eight (28 APOE-ε4+, age($M \pm SD$)=64.45±7.36; 30 APOE-ε4-, age($M \pm SD$)=65.23±10.34) participated in a 14-days-long actigraphy session supplemented by sleep diary. Thirty-five individuals (18 APOE-ε4+, age($M \pm SD$)=64.21±8.58; 17 APOE-ε4-, age($M \pm SD$)=65.00±9.54) underwent a 2.5-days-long laboratory session in dim light condition(<10lux) and followed a modified constant routine protocol in the Sleep and Brain Research Unit. After a baseline night, participants were randomly assigned to either a 40-h sleep deprivation(SD) or a multi-nap(MN) experimental condition followed by a recovery night. Cognitive assessments were administered every 4-hours. Nine 80-minute-long naps were scheduled every 160 minutes(MN condition).

RESULTS and DISCUSSION

Our results suggest that APOE-ε4 carriership in healthy elderly adults has a *limited* impact on subjective and objective sleep quality, daytime sleepiness and circadian rhythmicity measures besides a decrease in circadian rest-activity amplitude and a marginal decrease in the percentage of Total-Sleep-Time spent in N2 at baseline night. Yet, recovery sleep revealed an altered physiological recovery process in APOE-ε4 allele carriers that was reflected as a low percentage of deep sleep following SD protocol. Further, the outcomes suggest that spatial navigation performance is modulated neither by time-of-a-day nor is affected by increasing sleep pressure or by their associations with APOE-ε4 carriership.

Abbreviations

AD – Alzheimer's Disease

aMCI – amnestic Mild Cognitive Impairment

APOE – Apolipoprotein E

Arg – arginyl

Aβ – amyloid–beta

BBB – brain blood barrier

CCI – Cognitive Change Index questionnaire

COGT – cognitive test battery assessed during the sleep lab sessions

Cry – cryptochrome

CSF – cerebrospinal fluid

Cys – cysteinyl

DNA – deoxyribonucleic acid

EC – entorhinal cortex

ECG – electrocardiography

EEG – electroencephalography

EMG – electromyography

EOG – electrooculography

ESS – Epworth Sleepiness Scale

FC – functional connectivity

fMRI – functional magnetic resonance imaging

GABA – Gamma-Aminobutyric Acid

GAD-7 – Generalized Anxiety Disorder questionnaire

HVLT-R – Hopkins Verbal Learning Test-Revised

IS – Interdaily Stability

ISI – Insomnia Severity Scale questionnaire

IV – Intra-Daily Variability

kAR – Activity-to-Rest probability

KDT – Karolinska Drowsiness Task

kRA – Rest-to-Activity probability

KSD – Karolinska Sleep Diary

KSS – Karolinska Sleepiness Scale

L_5 – 5 least active hours

LC – locus coeruleus

LOAD – Late-Onset Alzheimer's Disease

M_{10} – 10 most active hours

m-ACE – mini Addenbrooke's Cognitive Examination

MADRE – Mannheim Dream questionnaire

MCI – Mild Cognitive Impairment

MCTQ – Munich Chronotype Questionnaire

MEQ – Morningness-eveningness questionnaire

MESOR – Midline Statistic Of Rhythm

MN – MultiNap protocol

MWM – Morris water maze task

N_1, N_2, N_3 – non-rapid eye movement sleep 1,2,3

NREM – non-rapid eye movement sleep

PER_3 – PERIOD3 gene

PET – positron emission tomography

PGO – Ponto-geniculo-occipital waves

PHQ-9 – Patient Health Questionnaire

PSG – polysomnography

PSQI – Pittsburgh Sleep Quality Index

PVT – Psychomotor Vigilance task

qPCR – quantitative polymerase chain reaction

r_{rb} - Rank-Biserial Correlation

RA – Relative Amplitude

REM – rapid eye movement sleep

ROCF – Rey Complex Figure

RT – reaction time

SCN – suprachiasmatic nucleus

SD – sleep deprivation protocol

SDMT – Symbol Digit Modalities test

SE – sleep efficiency

SHQ – Sea Hero Quest

SQ – sleep quality

SWA – slow-wave activity

SWS – slow-wave sleep

TBI – total time in bed

TMT – Trail Making tests

TST – total sleep time

VAS – visual analogue scales

VR – virtual reality

VST – Virtual Supermarket task

VST-A – Virtual Supermarket task - Allocentric response

VST-E – Virtual Supermarket task - Egocentric response

VST-HD – Virtual Supermarket task – Heading direction

WASO – wake after sleep onset

YASA – Yet Another Spindle Algorithm

Conference presentations resulting from this PhD

A. Michalak, T. Garcia Vite, Z. Shabana, V. Grove, A. Mann, T. Conway, C. Dietrich, J. Tsigarides, N. Gill, I. Clark, A. Wagner, A.-M. Minihane, E. Mioshi, M. Hornberger, A. Lazar; "Sleep and circadian rhythmicity in healthy older adults at low and high genetic risk of Alzheimer's disease: a multi-method research study."; Sleep Europe 2022, Athens, Greece (oral presentation)

A. Lazar, A. Michalak, T. Garcia Vite, Z. Shabana, V. Grove, A. Mann, T. Conway, C. Dietrich, J. Tsigarides, N. Gill, I. Clark, A. Wagner, A.-M. Minihane, E. Mioshi, M. Hornberger; "Early sleep and circadian markers of Alzheimer's: The impact of APOE-ε polymorphism on sleep-wake regulation, brain activity and cognition in healthy older adults"; Sleep Europe 2022, Athens, Greece (oral presentation)

A. Michalak, Z. Shabana, T. Garcia Vite, V. Grove, A. Mann, T. Conway, C. Dietrich, J. Tsigarides, N. Gill, M. Hornberger, A.S. Lazar; "Sex differences in the detrimental effect of sleep restriction and the benefits of scheduled short naps on vigilance and cognition in healthy elderly adults."; World Sleep Congress 2022, Rome, Italy (poster)

A. Mann, A. Michalak, Z. Shabana, T. Conway, C. Dietrich, E. Mioshi, V. Grove, I.M. Clark, A.M. Minihane, M. Hornberger, A.S. Lazar; "Subjective sleep quality is the strongest predictor of mental and physical health independent of chronotype, sleep duration, APOE-ε4 carriership, age, sex, alcohol consumption, and retirement status in healthy older adults."; World Sleep Congress 2022, Rome, Italy (poster)

A. Michalak; "Significance of addressing sex bias in elderly adults in sleep and cognitive research."; NIHR Applied Research Collaboration East of England, Early Career Researcher event, 2022 (oral presentation)

A.Michalak, V. Grove, Z. Shabana, C. Dietrich, E. Mioshi, A.M. Minihane, M. Hornberger, A.S. Lazar; "Depressive symptoms and daytime sleepiness are stronger predictors of subjective cognitive decline than objective cognitive performance, APOE genotype and subjective sleep quality in elderly individuals"; 25th Congress of the European Sleep Research Society (e-poster)

A.Michalak, V. Grove, Z. Shabana, C. Dietrich, J. Tsigarides, N. Gill, E. Mioshi, A.M. Minihane, M. Hornberger, A.S. Lazar; "Sleep restriction effects on object-location-associative-memory performance: a randomized controlled sleep deprivation versus multinap study in healthy elderly at low and high genetic risk of Alzheimer's"; 25th Congress of the European Sleep Research Society (oral presentation)

A.Michalak, C. Dietrich, G. Coughlan, V. Grove, Z. Shabana, M. Hornberger, A.Lazar; „The impact of APOE-ε polymorphism on the interaction between sleep, circadian rhythmicity and spatial navigation in healthy elderly people"; Postgraduate Research Conference 2019, University of East Anglia, Norwich, England (oral presentation)

A.Michalak; "The nature and significance of early sleep and circadian disturbances for the onset and progression of dementia"; Postgraduate Education Conference 2018, University of East Anglia, Norwich, England (oral presentation)

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INTRODUCTION

Introduction arrangement

As the main objective of the thesis was to investigate the impact of the APOE-ε4 allele on sleep, circadian rest-activity patterns and their interactions with spatial navigation, the introduction is divided into three chapters. Chapter I focuses on the APOE genotype, its biology and its associations with late-onset Alzheimer's Disease. Chapter II gives an overview of sleep, circadian rhythmicity and their associations with APOE polymorphism. Chapter III concentrates on spatial navigation and how it is influenced by APOE polymorphism and sleep. The interrelationship between APOE-ε4 carriership, sleep, circadian rest-activity and spatial navigation represents a research gap that is addressed by the thesis.

CHAPTER I: APOE-ε4 status as the strongest known genetic risk factor for late-onset Alzheimer's Disease

1.1. Dementia – a public health priority

Significant improvement in medicine that prolonged human life expectancy contributes nowadays to an increasing number of people living with dementia making it one of the main public health priorities. The estimated number of people living with dementia in 2020 exceeded 50 million worldwide and is estimated to double every 20 years, reaching 82 million in 2030 and 152 million in 2050 (Alzheimer's Disease International, 2020). In 2021 the estimated amount of $355 billion was paid for health care, long-term care and hospice for Americans with dementia aged 65 and older, whereas in 2020, the cost of $256.7 billion was attributed to unpaid dementia caregiving (2021 Alzheimer's disease facts and figures, 2021). The approximate number of caregivers of people with dementia in 2021 was 11.2 million. Caregiving can impact the emotional, psychological and physical outcomes of a caretaker which further highlights the complexity and social extent of dementia.

1.2. Alzheimer's Disease - the most common type of dementia

Alzheimer's Disease (AD) is the most common type of dementia accounting for an estimated 60–80% of cases and is the fifth leading cause of death in Americans age 65 and older (2021 Alzheimer's disease facts and figures, 2021). Alzheimer's leads to degeneration of nerve cells and neuroinflammation due to aggregation of extracellular amyloid plaques (Aβ), intracellular neurofibrillary tangles and abnormal activation of microglia. These neurological changes lead to neuronal death, damage to brain tissue and consequently, progressive brain atrophy.

Alzheimer's disease continuum follows three stages, i.e., preclinical Alzheimer's disease that is an asymptomatic phase, prodromal Mild Cognitive Impairment (MCI) due to AD, where an individual presents subtle cognitive changes which do not affect everyday life activities and finally dementia due to AD characterized by symptoms interfering with daily life (Aisen et al., 2017). Importantly, not all of the individuals presenting AD biomarkers will develop MCI and AD dementia, further, not all MCI patients will transit to AD and some of them might even regain normal cognition (Knopman et al., 2006, Bennett et al., 2006, Alzheimer's Disease International, 2020).

In the preclinical, asymptomatic phase of AD, the biomarkers of AD such as elevated levels of amyloid-beta (Aβ) and hyperphosphorylated tau are detectable by positron emission tomography (PET) scans and in the examination of cerebrospinal fluid (CSF). Moreover, there is a decreased brain glucose metabolism seen in the PET scan (Mosconi, 2014). In the early phase, normal functioning (despite present AD biomarkers) is facilitated by compensatory brain ability which reaches its limits in MCI,

where the neurodegenerative changes are reflected as mild cognitive alterations such as memory and thinking problems. Dementia due to AD is described as *mild*, *moderate* and *severe* to reflect the degree of symptoms attributed to brain atrophy (Albert et al., 2011).

The early *brain* changes attributed to AD are unnoticeable to the person affected. This initial silent phase is followed by progressive cognitive decline, especially in the memory domain and eventually physical disability and death (Zvěřová, 2019). Among the first brain areas affected by AD are the hippocampus and entorhinal cortex (EC) which are mirrored by difficulties in creating new memories and recalling recent events. As long-term memory depends less on the hippocampus and more on the cortex, at the initial stages of AD, an individual can still recall early life events but may have problems with saying what was served for a dinner (a sign of episodic memory alterations) (Albert et al., 2011). Importantly, brain structures affected in the earliest stages of AD overlap significantly with key nodes of spatial navigation networks which make spatial navigation and orientation deficits promising early cognitive markers of AD, especially considering that they tend to occur before episodic memory symptoms (Coughlan et al., 2018).

Further, while AD dementia affects more brain regions and leads to progressive thinning of the cortex, an individual starts forgetting even long-term memories. Advancing neurodegeneration leads to semantic memory and language impairment, problems with estimating distance and three dimensions that can increase the risk of falls (Albert et al., 2011, Alzheimer's Society). Damage to the frontal lobes impairs decision-making and executive functions. Procedural memories, e.g., playing an instrument often hold the longest.

On the *behavioural* level, at the early stage of AD, an individual might have problems with recalling recent conversations or names along with experiencing apathy and depression. While the neurodegeneration advances, communication and judgment become impaired, individuals become disoriented, confused and neuropsychiatric symptoms occur (such as agitation, suspiciousness or psychosis). Advanced clinical symptoms include speaking, swallowing and walking difficulties (2021 Alzheimer's disease facts and figures, 2021). In the mild stage of AD, the patients might still function independently but require assistance, in the moderate one, more help can be required to do basic activities such as bathing or cooking, finally, in the advanced stage, the individual requires constant care around-the-clock (Albert et al., 2011).

The disease duration and its phases vary significantly across individuals and are influenced by biological sex, APOE status and other factors such as CSF tau (Vermut et al., 2019). The studies showed that on average individuals age 65 and older survive from four to eight years from Alzheimer's dementia diagnosis, however, some studies reported patients living with AD dementia for even 20 years

(Alzheimer's Disease International, 2020). The Early-Onset (familial) AD[1] occurs between a person's 30s to mid-60s, whereas the Late-Onset (sporadic) AD starts after the age of 65 in over 90% of cases (Prince et al., 2013). Importantly, brain changes caused by AD pathophysiology can begin 20 or more years before clinical symptoms appear (Beason-Held et al., 2013) which gives a wide time window to introduce disease-modifying interventions.

Currently, Alzheimer's is an untreatable condition. Commonly used prescription drugs such as Acetylcholinesterase inhibitors (e.g. Donepezil), glutamate receptor antagonists (e.g. Memantine) and medication treating Behavioural and Psychological Symptoms of Dementia[2] help to manage the symptoms of AD. In the US, on the 7th of Jun 2021, the Food and Drugs Administration approved Aducanumab, as it is suggested that this medication *may* reduce amyloid deposits in the brain that can potentially slow down the progression of AD (Planche et al., 2021). Besides medications, Cognitive Stimulation Therapy or cognitive rehabilitation can be beneficial for individuals with AD. Support groups can help caregivers to find emotional support and assistance to manage emotional and physical stress related to caretaking.

1.2.1. Modifiable and non-modifiable risk factors of Alzheimer's

The biggest *non-modifiable* risk factors for AD are older age, family history of Alzheimer's and genetics, especially carriership of the APOE-ε4 allele (Hebert et al., 2010, Saunders et al., 2013, Farrer et al., 1997, Green et al., 2002). Individuals who have a first-degree relative (parent or sibling) with Alzheimer's are at higher risk to develop AD compared to those who do not (Green et al., 2002, Loy et al., 2014). The risk increases with the number of first-degree relatives with AD (Lautenschlager et al., 1996). Further, the female sex was found to also increase the risk of AD (Arnold et al., 2020). In the US, more than 60% of 5.3 million people diagnosed with AD were women (Arnold et al., 2020). These sex differences can be attributed to menopausal changes and estrogen loss which occur, on average, around the age of 51 (McKinlay et al., 1992). On the other hand, gender inequalities, especially in the first half of the 20th century could have led to lower educational attainment among women increasing their risk of dementia[3] (Rocca et al., 2014). Taken together, major risk factors for AD, i.e., age, APOE-ε4 genotype and female sex have a significant impact on metabolism which supports the view that AD is a metabolic

[1] The *Early Onset familial AD* is caused by mutations in three genes, i.e., APP, PSEN1, and PSEN2 that are inherited in Mendelian dominant fashion.

[2] The Behavioural and Psychological Symptoms of Dementia are especially common in later stages of AD and might include: elevated agitation, anxiety, wandering, aggression, delusion and hallucinations (Cerejeira et al., 2012).

[3] Low number of years of education was proposed to be a risk factor for AD, while higher educational attainment was suggested to facilitate building up cognitive reserves which allow the brain to counter the neurological damage more effectively (Larsson et al., 2017).

disease (Arnold et al., 2020). Metabolic dysfunctions such as abnormal glucose metabolism, insulin resistance and abnormal appetite regulation are known as comorbid disorders with AD (Cai et al., 2012).

Besides risk factors that cannot be changed, such as age, biological sex, genetics and family history, there are *modifiable risk factors* that can be adapted to lower the risk of dementia. Among possibly modifiable ones are years of education achieved in early life (<18 years), hypertension, obesity, hearing loss and Traumatic Brain Injury (TBI) in midlife (45-65 years) and smoking, physical inactivity, social isolation, depression, diabetes and air pollutions in later life (<65 years) (Livingston et al., 2020). These twelve modifiable factors are believed to potentially prevent or delay up to 40% of dementia cases. Further, modifiable factors, such as years of early life education, having a mentally stimulating career in midlife, taking part in leisure activities as well as having a good social network in late life were shown to decrease the risk of developing dementia in APOE-ε4 carriers who are at greater genetic risk of developing AD (Dekhtyar et al., 2019). The cited study suggests that lifelong engagement in *cognitive reserves* enhancing activities can lessen the risk of developing dementia in ε4 allele carriers.

Notably, none of the mentioned risk factors is *causal* and sufficient to cause AD. Alzheimer's has multicausal aetiology and the risk factors range from intracellular through psychosocial to environmental dimensions and *precise* associations between them are not established yet (Uleman et al., 2020). Therefore, system thinking using group model building[4] was applied to create the *systemic causal loop diagram* for AD providing a holistic and interdisciplinary approach describing how different risk factors *might* interact with each other and the underlying biological structures leading to cognitive decline due to AD (Uleman et al., 2020, Figure 1). For instance, depressive symptoms (that are a prominent risk factor of AD) are involved in several feedback loops, i.e., cognitive functioning, sleep quality, experienced stress and social interactions. Many of these factors create even more loops among each other. Importantly, the models point out several modifiable risk factors which could be considered in policymaking, e.g., allocating more funding to combating loneliness. On the other hand, the APOE-ε4 genotype which is a non-modifiable risk factor might play a role in dyslipidemia (unhealthy levels of fats in the blood) and therefore contribute to AD pathogenesis. Importantly, dyslipidemia can be usually managed by promoting a healthy lifestyle and medications (i.e., statins and fibrates).

[4] During the Group Model Building process, 15 experts from multiple disciplines within AD research, complexity research and computational modelling discussed the difference between cognitive decline trajectories in sporadic AD compared with normal aging, starting from midlife.

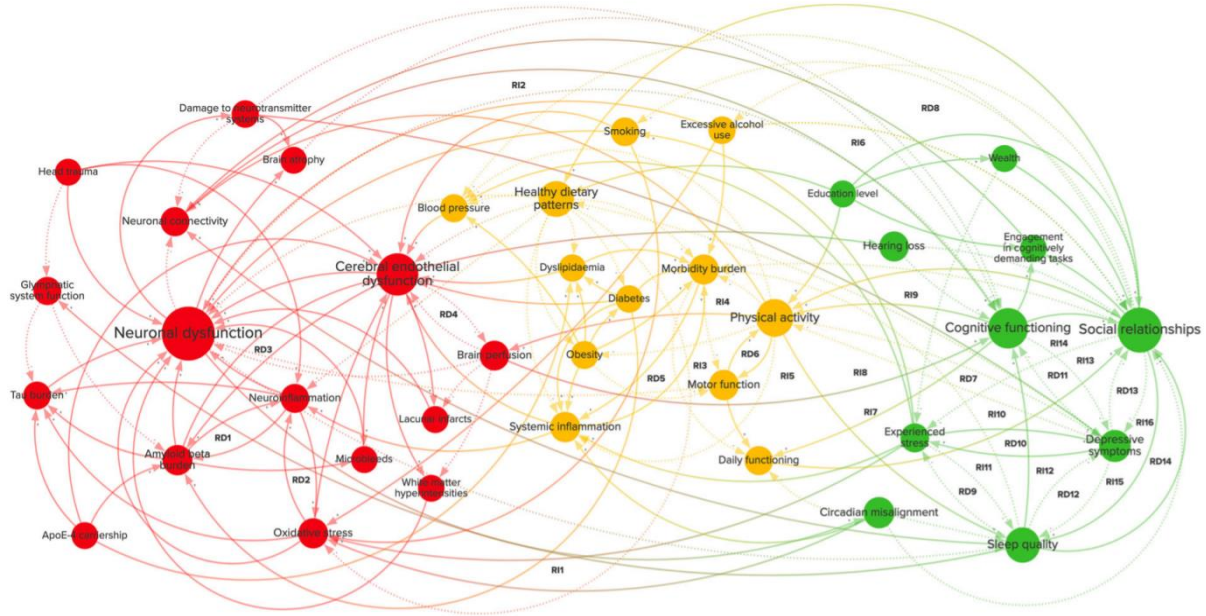


Figure 1. The causal loop diagram for sporadic Alzheimer's Disease (Uleman et al., 2020). Variables associated with brain health are drawn in red, physical health, in yellow and psychosocial health in green. Solid lines with '+' show a positive connection, i.e., an effect in the same direction, whereas dotted lines with '-' an effect in the opposite direction. Source of the diagram: Uleman et al., 2020

1.3. APOE-ε4 - the strongest genetic risk factor for Alzheimer's disease

1.3.1. Biology of Apolipoprotein E

The APOE gene (locus: 19q13.31) provides instruction for the formation of a protein called apolipoprotein E, known as ε2, ε3 and ε4. Three APOE isoforms differ by a single amino acid, i.e., arginyl (Arg) and cysteinyl (Cys) at positions 112 and 158 in the 299-amino-acid-long sequence, where, **ε2:** Cys112, Cys158, **ε3:** Cys112, Arg158 and **ε4:** Arg112, Arg158 (Zhong& Weisgraber, 2009). There are six combinations of the codominant alleles associated with this two single-nucleotide polymorphisms, i.e., ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4. This single amino acid polymorphism affects the structure of APOE isoforms and modulates binding properties to lipids, receptors and Aβ (Frieden & Garai, 2012).

Apolipoprotein E (APOE) plays a critical role in supporting injury repair and redistributing cholesterol and other lipids to neurons via binding to the APOE receptors on the cell surface (Liu et al., 2013, Yamazaki et al., 2019). In the Central Nervous System, APOE is richly expressed by astrocytes, microglia, vascular mural cells and choroid plexus cells, whereas in the periphery by hepatocytes and macrophages (Kang, et al., 2018)[5]. Most APOE is synthesized in the central nervous system, which is

[5] *astrocytes* - glia delivering energy to neurons and managing inflammation; *microglia* - macrophages of CSN; *vascular mural cells* - fundamental components of brain blood vessels; *choroid plexus cells* - cover capillary loops responsible for the production of CSF; *hepatocytes* - major parenchymal cells in the liver; *macrophages* - type of white blood cells that help eliminate foreign substances

supported by the fact that the APOE genotype has not changed to the donor's genotype following a liver transplant (Linton et al., 1991).

1.3.2. APOE status and Alzheimer's Disease

APOE-ε4 carriership is the strongest genetic risk factor for sporadic, i.e., Late-Onset Alzheimer's Disease (LOAD) (Yamazaki et al., 2019). Compared to ε3 homozygous, individuals who are carriers of one copy of the ε4 allele have ~3-times higher risk of developing LOAD, whereas in the case of ε4 homozygotes, the risk increases from 8 to 12-fold (Corder et al., 1993, Loy et al., 2014, Holtzman et al., 2012, Michaelson, 2014). APOE-ε4 allele also accelerates the onset of LOAD, by 2-5 years in heterozygotes and 5-10 years in homozygotes (Corder et al., 1993, Sando et al., 2008). Conversely, the APOE-ε2 allele appears to be protective against AD. Being a carrier of one allele of ε2 was shown to decrease the risk of AD (Odds Ratio = 0.6) relative to the APOE-ε3/ε3 (Farrer, 1997). APOE-ε3/ε3 genotype serves commonly as a reference group because of its prevalence (more than 60% in the general population) and neutral risk to the development of AD (Alzgene, 2010).

The frequency of the ε4 allele among AD patients is prominent (Figure 2). According to the meta-analysis involving 20 studies that assessed the frequency of APOE polymorphism in AD patients, 56% of Americans diagnosed with AD had one copy of the APOE-ε4 allele, whereas 11% were homozygotes (Ward et al., 2012). The highest frequency of ε4 status was found in Northern Europe, where 61.3% of AD patients had one copy of the ε4 allele, whereas 14.1% had two (Ward et al., 2012). Importantly, studies showed that the distribution of APOE isoforms varies ethnically, for instance, a higher frequency of the ε4 allele is present among African Americans than among Euro-Americans (Rajan et al., 2017, Evans et al., 2003, Tang et al., 1998). It was also reported that the effect of APOE status on cognition varies across different races (Beydoun et al., 2021).

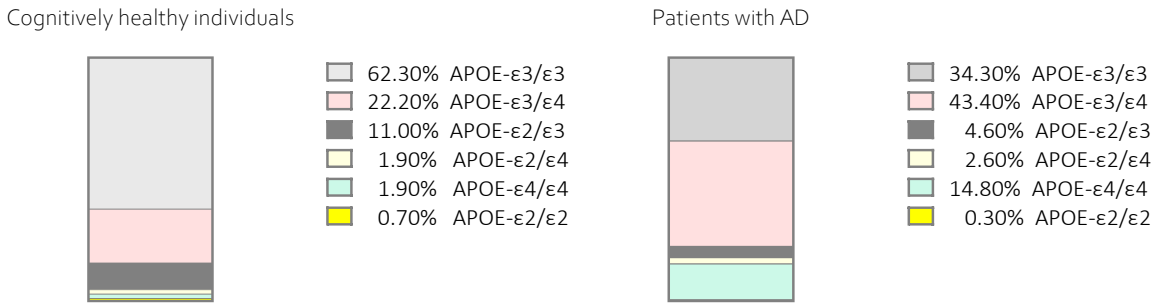


Figure 2. Frequency of APOE genotyping in cognitively intact adults and patients with Alzheimer's Disease. Figures are based on Alzgene, 2010

The association between APOE-ε4 carriership and AD is not causal. Yet, APOE-ε4 was shown to be associated with key AD-related clinical features such as the increased risk of late-onset AD, proatherogenic changes in lipoprotein distribution, elevated risk of cerebral amyloid angiopathy, increase risk of tau pathology in AD, reduced cerebral glucose metabolism, worse synaptic pathology in AD patients, increased risk of vascular cognitive impairment and pathologies contributing to neurovascular dysfunctions and mitochondrial dysfunction (Genin et al., 2011, Phillips, 2014, Rannikmae et al., 2014, Tiraboschi et al., 2004, Jagust et al., 2012, Koffie et al., 2012, Davidson et al., 2006, Chen et al., 2011). APOE-ε4 is also associated with cardiovascular disease and diabetes mellitus (Liu et al., 2019). It was shown that ε4 allele carriers are at increased risk of cardiovascular disease and diabetes mellitus and present a higher level of lipid profiles in the blood (Liu et al., 2019). Importantly, diabetes mellitus and hypertension which are prime risk factors for cardiovascular disease were indicated as the modifiable risk factors of AD (Livingston et al., 2020).

APOE-ε4 affects several AD-related pathogenic pathways increasing the risk of AD relative to APOE-ε3/ε3 through *a toxic gain of function* and *loss of physiological normal function*, i.e., decreased Aβ clearance, increased Aβ accumulation and Tau-mediated neurodegeneration, decrease in insulin signalling, glucose metabolism, cerebrovascular and synaptic functions, Brain Blood Barrier (BBB) integrity, and lipid transport what leads overall to altered neuronal injury repair (Figure 3, Yamazaki et al., 2019).

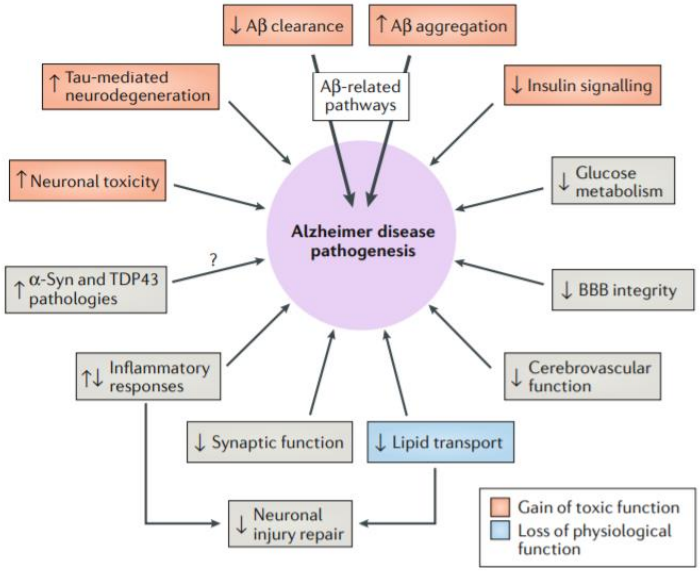


Figure 3. Involvement of APOE-ε4 in Alzheimer's Disease (AD) pathogenesis. Relatively to APOE-ε3/ε3, APOE-ε4 impacts adversely the efficiency of several brain homeostatic pathways, including lipid transport, synaptic function, glucose metabolism, cerebrovascular function and blood brain barrier integrity as well as microglia responsiveness. Red boxes show pathways that elevate the risk of AD via the *gain of toxic function*. Decreased efficiency in lipid transport mirrors the *loss of physiological function*. Elevated aggregation and impaired clearance of Aβ are the major pathways by which ε4 carriership affects AD pathogenesis. Image source: Yamazaki et al., 2019

APOE status influences AD pathogenesis *primarily* through Aβ clearance and aggregation. Immunohistochemical studies showed that APOE is a protein component of Aβ plaques, Tau tangles and cerebral vessels that binds and modulate the Aβ accumulation in an isoform-dependent manner, i.e., ApoE4 > ApoE3 > ApoE2 (Namba et al., 1991, Strittmatter et al., 1993). APOE-ε2 isoform is the most effective with Aβ clearance, while APOE-ε3/ε3 deals with Aβ clearance decently enough to be out of clinical interest. On the other hand, non-demented APOE-ε4 carriers were shown to have a 1 to 3 times higher prevalence of cerebral amyloid pathology compared to non-carriers suggesting less efficient Aβ clearance (Jansen et al., 2015).

Additionally, it was reported that APOE-ε4 allele carriership triggers the breakdown of the BBB, (Montagne et al., 2021). Notably, carriers of the ε4 allele can be distinguished from ε3 homozygotes based on the degree of impaired integrity of BBB in the hippocampus and temporal lobe (Montagne et al., 2021). The damage to vascular cells forming BBB (that seems to be accelerated by activation of inflammatory response in the blood vessel) was associated with more pronounced cognitive dysfunction in APOE-ε4 carriers. The effect was not related to Aβ or tau pathology which suggests that the BBB dysfunction contributes to APOE-ε4-associated cognitive decline independently of AD. Furthermore, vascular changes in the eye, i.e., lower retinal capillary densities[6] in cognitively healthy APOE-ε4 carriers (compared to non-carriers) suggest that disease of small vessels can be another early marker of AD (Elahi et al., 2021).

Notably, a recently developed algorithm can determine when cognitively intact individuals will develop AD (Schindler et al., 2021). The inputs for the algorithm are the age of an individual and one PET scan with amyloid levels based on which the model can estimate the progression of neurodegeneration (*if any*) and how much time is left before a cognitive decline due to AD will manifest. According to the authors, Aβ aggregation has *a tipping point in* which the time point (age) varies greatly across individuals. Once the tipping point is reached, the accumulation of Aβ follows a well-known pattern. The study reported that APOE-ε4 allele carriers reached the tipping point earlier, i.e., by 10 years in the case of ε4 homozygotes compared to non-carriers.

1.3.3. Age-dependent effect of APOE polymorphism on cognitive performance

The impact of the APOE genotype on cognition was shown to be manifested in an age-dependent manner (Rawle et al., 2018). During ageing, in comparison to ε3 homozygotes, ε4 allele carriership accelerates cognitive impairment, while the APOE-ε2 allelic variant contributes to less cognitive decline and as revealed by animal study, the association is independent of Aβ accumulation

[6] Retinal capillary densities were considered in the study as proxies for brain capillaries.

and age-related neuroinflammatory alterations (Shinohara et al., 2016). Another study reported that elderly ε2 carriers (ε2/ε2 and ε2/ε3) maintained the level of their verbal learning performance in 3-years-follow-up, which was not a case for other APOE genotypes, where a decline in performance was pronounced (Helkala et al., 1996). Conversely, relative to APOE-ε3/ε3, ε4 allele carriership was associated with quicker cognitive decline in episodic memory, executive function, language, and attention, moreover, the reported discrepancies tend to rise with age (Salmon et al., 2013). A meta-analysis including 76 publications focused on the effects of APOE on cognition across a lifespan suggested that the ε4 allele has a small but significant detrimental effect on episodic memory, global cognitive performance, executive functioning, and perceptual speed, moreover, the discrepancy with non-carriers increases with age (Wisdom et al., 2011). The effect of the APOE-ε4 allele on cognitive decline was shown to be dose-dependent, i.e., it is faster in homozygous than heterozygous (Rawle et al., 2018).

The study based on a large population sample (n=6,560) that assessed episodic memory, working memory, mental speed, reaction time and reading vocabulary suggested that in early life (defined as 20 to 64 years of age) ε4 allele does not have a preclinical value from a cognitive performance perspective (Jorm et al., 2007). However, a recent longitudinal study reported that APOE-ε4/ε4 carriers, compared to non-carriers had a faster rate of decline in memory between ages 43 and 69 which suggest a slightly faster rate of episodic memory decline from midlife to early old age (Rawle et al., 2018).

1.3.4. APOE and cognitive and brain reserves

Age-related cognitive impairment can be overshadowed by *cognitive reserves* which refer to the brain's ability to use existing cognitive resources or compensatory mechanisms to perform a given task. Educational attainment is a commonly studied proxy measure of cognitive reserves (Wang et al., 2020). On the other hand, *brain reserves* describe organic changes that can enhance tolerance to pathology in the brain itself (Stern et al., 2012).

A recent study showed that educational attainment predicts greater brain reserve and that cortical volume (especially the integrity of the precuneus cortex and superior frontal gyrus) can alleviate risk factors associated with ε4 allele carriership (Coughlan et al., 2021). Additionally, in cognitively healthy ε4 carriers, educational attainment was correlated with a higher volume of precuneus. Interestingly, ε4 homozygotes who performed better at the episodic memory task had also higher volumes of precuneus, while ε4/ε4 carriers with cognitive impairment had lower precuneus volumes which nicely highlights an important contribution of this structure to brain reserves. The study

highlighted an interesting research direction as it is not known how these neuroimaging markers of brain reserves in at-risk individuals (ε4 allele carriers) interact with Aβ and tau burden and the onset of AD pathology.

Furthermore, lifelong engagement[7] in *cognitive reserves enhancing activities* such as education, work complexity, social network, and leisure activities was reported to lessen the risk of developing dementia in ε4 allele carriers (Dekhtyar et al., 2019). Notably, the cognitive reserves[8] seem to mitigate the genetic disadvantage attributed to APOE-ε4 carriership (Figure 4). Higher cognitive reserves were also associated with a reduced risk of transition from normal cognition to symptomatic AD in ε2 carriers relative to non-carriers (Pettigrew et al., 2013).

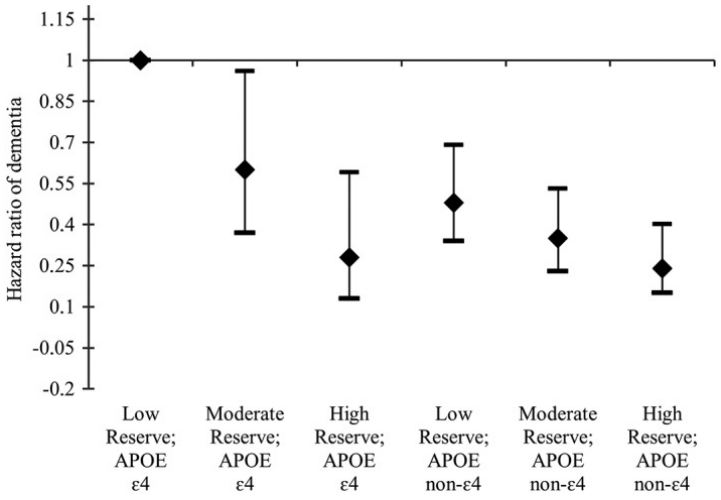


Figure 4. The effect of the cognitive reserve indicator and ε4 allele carriership on the risk of dementia. The risk of dementia in APOE-ε4 carriers and non-carriers was reduced accordingly with a level of *cognitive reserves indicator*[9]. Notice that the risk of dementia between high reserve ε4 carriers and high reserve ε4 non-carriers is at almost the same level. Source of figure: Dekhtyar et al., 2019

[7] The study's participants were 2,556 cognitively intact individuals aged ≥60 years from the ongoing prospective community-based Swedish National Study on Aging and Care.
[8] *Cognitive reserves composite score* consisted of years spent in education, reading, and vocabulary.
[9] *A cognitive reserve indicator* was calculated based on four validated contributors, i.e., early life education, midlife substantive work complexity, late life leisure activities, and late life social networks.

SUMMARY – APOE-ε4 allele carriership and risk of sporadic Alzheimer's Disease

APOE genotype is involved in lipid metabolism along with numerous functions in the central nervous system which efficiency follows an isoform-dependent manner. APOE-ε4 carriership is the biggest genetic risk factor for developing Late-Onset Alzheimer's Disease. APOE-ε4 affects several AD-related pathogenic pathways increasing the risk of AD through *a toxic gain of function* and *loss of physiological normal function*. The effect of isoforms on cognitive decline seems to get stronger with age and was shown to be also modulated by cognitive and brain reserves. Nevertheless, despite numerous studies, it is still unclear how *exactly* ε4 allele carriership increases the risk of sporadic AD. This, in turn, slows down the development of treatments targeting specific APOE-ε4 pathways contributing to AD and exacerbating its progression.

As shown by the Causal Loop Diagram for sporadic Alzheimer's Disease, AD is a multifactorial disease, hence, each risk factor and its relationship with other factors deserve attention. The biology of APOE isoforms provides an interesting research angle, thus, knowledge gaps related to APOE polymorphism should be addressed to facilitate a multidirectional effort to understand the complexity of AD. Therefore, the presented PhD project aims to investigate for the first time the impact of the APOE-ε4 allele, time of the day and experimentally manipulated sleep pressure on spatial navigation performance. Accordingly, Chapter II covers the relationship between APOE polymorphism, sleep and rest-activity patterns, whereas, Chapter III focuses on the impact of APOE polymorphism on spatial navigation and its associations with sleep.

CHAPTER II. Sleep and Circadian Rhythmicity

2.1. What is sleep and how do we study its mysteries?

Sleep is a naturally occurring reversible state of unconsciousness, characterized by behavioural quiescence, reduced level of responsiveness and relative sensory-motor disconnection from the environment. In mammals, there are three vigilance states, i.e., wakefulness, non-rapid eye movement sleep (NREM) and rapid eye movement sleep (REM). Sleep is modulated by two physiological mechanisms, i.e., *sleep homeostasis* which promotes sleep/wakefulness according to the previous sleep/wake history and *circadian rhythmicity* which regulates the sleep-wake cycle and repeats roughly every 24 hours (Borbély et al., 1982). Sleep and rest-activity patterns can be measured via objective (polysomnography, actigraphy, hormone fluctuations, e.g., melatonin, cortisol, body temperature) and subjective measures (questionnaires).

2.1.1. Polysomnography

Sleep *quantity*, i.e., duration of total sleep and its stages and *quality*, i.e., latency to sleep, brief arousals after sleep onset, and amount of slow waves are assessed by measuring electrical brain activity using electroencephalography (EEG). Combining EEG with ocular (electrooculography, EOG) and muscular activity (electromyography, EMG) allows sleep stage classification. Sleep staging follows worldwide used sleep staging manuals, i.e., A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects (Rechtschaffen &Kales, 1968) and the American Academy of Sleep Medicine (Berry et al., 2020). Polysomnography (PSG) which combines several sensors[10], besides being an objective scientific tool to investigate sleep is also an approved medical procedure to diagnose sleep disorders.

In sleep research, EEG electrode placement follows usually[11] internationally recognized, standardized American Electroencephalographic society's international 10-20 system of the EEG placement method (Sharbrough et al., 1991), which ensures proportional inter-electrode spacing and appreciates the size and shape of the participant's skull. Further, EEG power spectrum analysis measuring the power of specific frequency bands provides more detailed information about sleep quality. One can analyse also EEG topography to investigate local correlates of sleep such as K-complexes, sleep spindles or slow waves (e.g., Ioannides et al. 2019, Laurino et al. 2019, Alfonsi et al., 2019).

[10] PSG consists of: EEG, EOG, electrocardiography (ECG), EMG (chin EMG and leg EMG), breathing effort belts, pulse-oximetry, snoring sensors, microphone and nasal cannula (registering airflow).
[11] For instance, high-density, 256 electrodes cap from Ant Neuro does not follow 10-20 system of electrodes spacing.

2.1.2. Actigraphy

Another commonly used non-invasive objective method to investigate sleep and rest-activity patterns is actigraphy. The actigraphy is cost-effective and ecologically valid as it allows the recording of habitual rest-activity patterns in the participant's natural environment. The actigraphy outcomes were shown to be strongly correlated with entrained endogenous circadian rhythms (Ancoli-Israel et al., 2003). From a medical perspective, actigraphy provides information about potential circadian disorders, insomnia and nocturnal awakenings. Actigraphic data are recorded via an actiwatch device. The device is implemented with a light sensor and is worn usually on the non-dominant wrist to record gross motor movements using a tiny built-in accelerometer. The recording is usually worn for several days which allows for visualizing the daily pattern of rest and activity. Actigraphy recording allows for defining approximate rest (sleep) and activity (wake) periods. Actigraphy is usually accompanied by a *sleep diary* where the participant is asked to provide information about bedtime, waketime, number of awakenings, levels of sleepiness before and after sleep, quality of sleep, dreams recall and physical activities.

2.1.3. Other common measures

Hormones such as melatonin and cortisol which can be extracted from either saliva or blood are reliable indicators of the intrinsic circadian clock as both of them show characteristic patterns of activity over 24 hours. Moreover, core body temperature and alertness follow distinctive, circadian patterns. The gold standard cognitive test measuring fluctuation of vigilant attention is the Psychomotor Vigilance Task (PVT) (Dinges & Powell 1985) which is a monotonous and lengthy reaction time task (time of the assessment varies usually between 5 to 15 minutes depending on the study protocol) which is very sensitive to changeable levels of vigilance.

2.2. Sleep architecture

Sleep is heterogeneous from a physiological standpoint (Figure 5). During sleep, EEG, EOG and EMG patterns change along with sleep deepening (Table 1). We can distinguish Non-Rapid eye movement sleep (NREM, N) consisting of three phases called N1, N2 and N3 and Rapid eye movement sleep (REM, R). During typical night sleep (assuming around 8h of sleep), a young adult spends about 2% to 5% in N1 (i.e. transition between sleep and wake), 45% to 55% of the time in N2 (superficial sleep), 10% to 20% in N3 (deep sleep) and 20% to 25% in REM sleep (Carskadon & Dement, 2017). On average, NREM-REM cycles are about 90-110 minutes long and recur four to five times during the night. The distribution of the sleep stages changes overnight. N3 is generated mostly in the first third of the night and its length expresses sleep need (sleep homeostasis), whereas, REM sleep is the most prominent in the last third of the night and is linked to the circadian rhythm of the core body temperature (Czeisler et al., 1980). Volitional control, length of prior sleep and genetic sleep need is the most significant factor

in the length of sleep in young adults. Age is the strongest factor in altering sleep stage distribution across the night in healthy adults (Mander et al., 2017).

Dream recall is reported in about 80% of arousals from REM sleep (Foulkes, 1962). Importantly, dreams are not an exclusive occupant of REM sleep and the conscious experience can be recalled during N1, N2 and N3 sleep (e.g., Schredl, 2007). Importantly, an individual will be more alert when woken up from REM sleep compared to NREM which is in line with the hypothesis that the function of REM sleep is to stimulate the brain following inactivity and metabolic slowness related to NREM sleep.

Sleep stage	electroencephalography (EEG)	electromyography (EMG)	electrooculography (EOG)
N1	Low-amplitude, mixed frequency activity in the range of 4-7 Hz, less than 50% alpha activity	The chin EMG amplitude is variable, often lower than during wake	Slow-rolling eye movements, possibly asynchronous
N2	Stage 2 sleep is characterised by the presence of one or more K-complexes (that are not correlated with arousal) and sleep spindles (11-14Hz)	Usually no motion artefacts. The chin EMG is of variable amplitude, but usually lower than during Wake	Usually, eye movement is not noticed during N2
N3	Slow-wave sleep. Minimum 20% of slow wave activity, i.e., waves of 0.5 – 2 Hz frequencies with a peak-to-peak amplitude of ≥75μV in the frontal derivation. Sleep spindles might be seldom seen also during N3.	The chin EMG is of variable amplitude, often lower than in stage N2, and sometimes can be as low as in REM sleep.	Usually, eye movement is not noticed during N3
REM	Stage REM sleep is characterised by a low-voltage, mixed-frequency EEG, Sawtooth waves also may be present	low-amplitude chin EMG – baseline EMG activity, usually at the lowest level of all recording	Conjugated, episodic, sharply peaked, irregular eye movements

Table 1. Physiological characteristics of sleep stages according to the American Academy of Sleep Medicine manual version 2.6 (Berry et al., 2020). Abbreviations: *N1*, *N2*, *N3* - non-rapid eye movement sleep 1,2,3 ; *REM* - rapid eye movement sleep

2.3. Definition of sleep stages

2.3.1. Non-rapid eye movement

NREM1 (N1) occurs mostly at the beginning of the sleep cycle (Berry et al., 2020). In this stage, the alpha rhythm characteristic for relaxed closed-eye wakefulness is gradually suppressed and replaced by low-amplitude theta oscillations with the occurrence of vertex spikes (Berry et al., 2020). N1 initiation is accompanied by slowing the heart rate, reduction of muscle tone and characteristic slowly rolling eye movements. Sleep onset is commonly accompanied by hypnic jerk which is a localized muscle contraction (Berry et al., 2020). N1 is a transitional state between wakefulness and sleep, therefore it is characterized by a low awakening threshold. N1 sleep can be easily discontinued by calling a person by name, light touch or the sound of a gently closing door. N1 is a transitional state during the night and its increased prevalence indicates fragile and disturbed sleep. N1 is highly heterogeneous from an electrophysiological perspective and the transition from wakefulness to N1 sleep might be traced by Hori's nine-stage EEG system (Tanaka et al., 1996). The nine-stage scale shows the complexity of the transition from wake to sleep. Sleep is indicated by an occurrence of the first sleep spindle which is a hallmark of N2.

During NREM2 (N2), background theta activity is accompanied by K-complexes and sleep spindles which are hallmarks of stage N2 sleep. K-Complexes (KC) are the "*largest events in healthy human EEG*" (Cash et al., 2009), characterised by a double-phased wave with a high amplitude where the negative sharp wave is immediately followed by a positive component and lasts at least 0.5 seconds. They can emerge spontaneously or in a stimulus-induced manner (stimuli that lead to arousals during N1 often result in KC response in N2 without awakening, e.g., the calm sound of closing a window). The functional role of K-Complexes is not well established, however, low-level sensory processing (mainly acoustic) and sleep-protective mechanisms are the most supported ones (e.g., Halász, 2005; Jahnke et al., 2012; Ioannides et al., 2017, Blume et al., 2017). K-complexes are usually followed by sleep spindles.

Sleep spindles are composed of a group of rhythmic waves which progressively increase and then gradually decrease in amplitude (Berry et al., 2020). A sleep spindle is a burst of distinct waves with a frequency of 11-16 Hz (sigma band), most commonly 12-14 Hz with a total duration of ≥ 0.5 seconds. Spindles are further divided into slow (~11 – 13.5 Hz), and fast ones (~13.5 – 16 Hz) which mirror two distinct spindles generators with different brain topographies (De Gennaro & Ferrara, 2003, Schabus et al., 2007). Spindles are generated by the thalamus and are regulated by slow oscillations (Steriade, 2000, Molle et al., 2004). Spindles contribute to sleep maintenance associated with sensory gating, as well as memory consolidation (e.g., Urakami et al., 2012, Schabus et al., 2006). The density of sleep spindles was shown to be positively correlated with fluid intelligence tests and are reliably associated with

memory consolidation (e.g., Schabus et al., 2006). Further, precise Slow Oscillation–Spindle coupling is vital for sleep-dependent memory consolidation (Muehlroth et al., 2019, Hahn et al., 2020). The occurrence of the first sleep spindle is associated with a sudden and significant increase in the threshold of auditory perception, as well as a total loss of awareness of the surrounding (Strauss, 2015).

Slow-wave sleep (SWS), called also deep sleep is the third stage of NREM sleep (N3). The hallmarks of SWS are highly synchronised delta waves (1-4 Hz) of dual, thalamic and cortical components and slow oscillations (>1 Hz) of cortical origin (McGinty & Szymusiak, 2017). SWS is characterized by a widespread synchronization of cortical activity where neighbouring cortical neurons seem to tune agreeably. Every oscillation combines a depolarised *up* active state when neurons fire irregularly, followed by a hyperpolarized *down* silent phase. A higher number of slow waves indicates deeper NREM sleep, i.e., the highest awakening threshold (Cirelli, 2010). K-complexes and sleep spindles might be generated during SWS, however, to a considerably smaller extent compared to N2. Deep sleep has many important functions starting from tissue repair (supported by the release of growth hormone), through memory consolidation to the most efficient work of the glymphatic system (e.g., Payne & Walker, 2008, Walker, 2009, Xie et al., 2014). During SWS the space between neurones increases by about 60%, allowing the cerebral spinal fluid to remove neurotoxic waste that accumulates during wakefulness (Xie et al., 2014). SWS is also an indicator of sleep homeostasis, i.e., higher sleep pressure is correlated with more time spent in N3 (Dijk et al., 1990). The synaptic strength is reflected in the slope and amplitude of the slow waves (e.g., Fattinger et al., 2014).

2.3.2. Rapid Eye Movement sleep

Rapid Eye Movement sleep (REM) is characterized by rapid, sharply peaked eye movements, total muscle atonia and significantly increased EEG activity showing high-voltage theta waves generated by the hippocampus. REM sleep shows phasic and tonic phases (microstates) that have notably different neuronal states that affect environment alertness, generated spontaneous and evoked activity of the cortex and processing of information (Simor et al., 2020). *Phasis REM* is a microstate with rapid eye movements (increased EOG amplitude), while *tonic REM* refers to periods without eye movements (reduced EOG amplitudes).

REM sleep is generated by pons in the brainstem and adjacent areas of the caudal midbrain (Sigel, 2009). During REM sleep, pulse rate and sleeping pattern become irregular and cerebral metabolism mirrors the one in wakefulness. Hippocampal theta rhythms in the frequency between 4 to 7 Hz are a prominent hallmark of REM. Further, Ponto-Geniculo-Occipital waves (PGO) occur during the transition from NREM to REM sleep and during REM sleep itself (Sigel, 2009). PGO waves play an

important role in central nervous system maturation (Marks et al., 1995). Animal studies demonstrated that PGO waves are associated with learning and memory consolidation (Datta et al., 2004).

REM is also the stage of sleep when most dreams occur. In comparison to dreams during NREM sleep which are more logical and thought-like, during REM sleep oneiric content is illogical, ludicrous and often bizarre (Strauss, 2015). The arousal threshold is variable (due to alterations between tonic and phasic REM) which suggests that the arousing stimuli could be incorporated into the dream content without leading to arousal. Interestingly, the temperature of the environment can disturb REM sleep more prominently than NREM sleep which is attributed to the fact that mammals have very limited ability to thermoregulate during REM sleep, i.e., sweating or shivering can occur during NREM but are very constrained during REM sleep (Carskadon & Dement, 2017).

2.3.3. Dreams recall

There are within-person and between-person variations in dream recall (Zadra & Robert, 2012). According to Cohen's *theory of dream recall*, the more relevant, bizarre or emotionally loaded the dream was, the more likely it will be remembered (Cohen, 1974). Regarding, between-person differences, it was shown that recalling dreams gets worse with age, and is higher in women and among individuals who score higher on creativity scales (Nielsen, 2012, Vallat et al., 2022). Further, being interested in dreams was indicated as the best predictor of dream recall. High dream recallers compared to low dream recaller have increased brain activity to auditory stimuli during wakefulness and sleep and more intervals of intra-sleep wakefulness (Vallat et al., 2017) as well as higher baseline activity in the Default-Mode Network which is known for being active during mind-wandering and daydreaming (Eichenlaub et al., 2014).

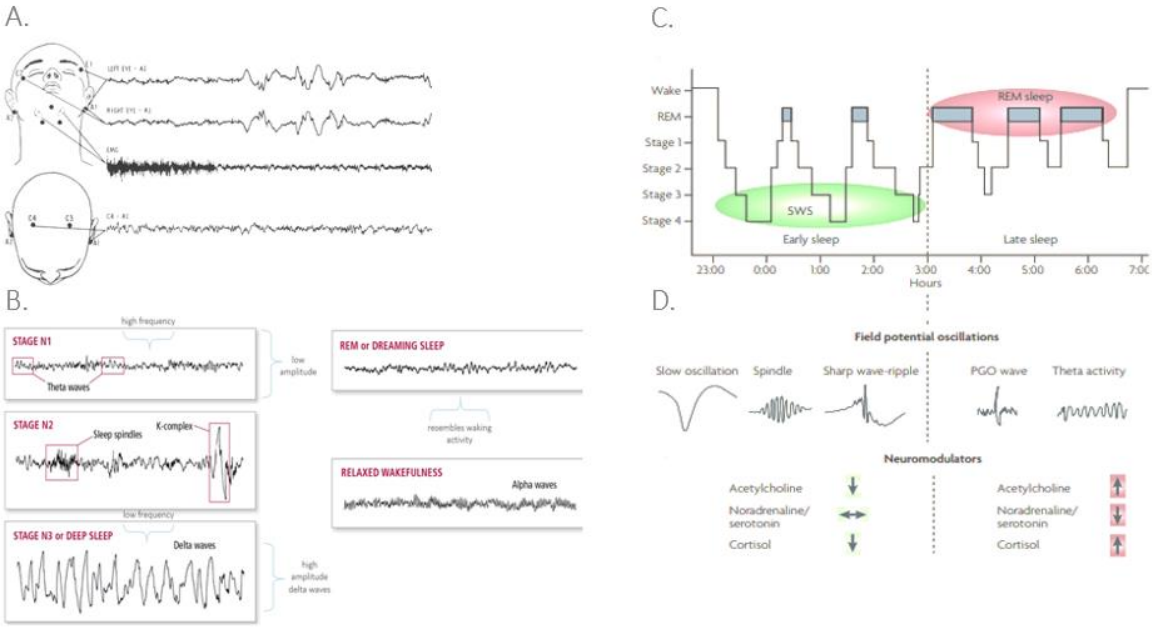


Figure 5. Sleep architecture and electrophysiological correlates of sleep and wakefulness. A. Sleep staging is based on the interpretation of EEG, EOG and EMG physiological changes. B. Each sleep stage is characterized by distinct electrophysiological features and different ratios of dominant frequencies, e.g., the hallmarks of stage NREM2 are K-complexes and sleep spindles with a mixed frequency background with a noticeable reduction of alpha activity compared to N1. C. Hypnogram is a graphical representation of sleep stages distribution throughout the sleep episode. SWS is a dominant stage during 1st part of the night (green highlight), whereas REM sleep (red highlight) occurs mainly in the 2nd half of the night. D. Slow oscillations, sleep spindles and sharp wave ripples are hallmarks of NREM sleep, whereas Ponto-Geniculo-Occipital waves and theta activity of REM sleep. Each sleep stage is characterized by a different mixture of neuromodulators, for instance, in REM sleep, the levels of acetylcholine and cortisol increase, whereas noradrenaline levels decrease (in comparison to NREM sleep). The image was adapted from Horne, 1988 and Rash & Born, 2013

2.4. Neuroanatomy of sleep

2.4.1. Neural systems underlying sleep and wakefulness

Contrary to its behavioural characteristics, sleep is a very active state from a neurobiological perspective. The sleep-promoting mechanisms are present at all the neuroaxis, i.e., the forebrain including the neocortex as well as the brainstem. There are four interacting known neural systems mediating sleep and wakefulness:

A) *a forebrain system* – promotes SWS by secretion of Gamma-Aminobutyric Acid (GABA) into the tuberomammillary nucleus located in the hypothalamus. Electrical stimulation of the basal forebrain promotes sleepiness, whereas its lesion causes insomnia (Clemente & Sterman, 1967).

B) *a brainstem system* – fosters wakefulness sending the signal from the reticular formation to the forebrain and cerebral cortex. The reticular formation originates in the core of the brainstem and projects to the thalamus and cerebral cortex. Due to its thalamus-cortex connections, the

formation takes part in sensory processing during sleep. Injury to this region can lead to irreversible coma, whereas the lesion in animals causes persistent sleep.

C) *a pontine system* – triggers REM sleep via the activity of the subcoeruleus which projects to medullary axons which in turn project to the spinal cord to suppress motor neurons (muscle atonia). Electrical stimulation or pharmacological intervention with cholinergic antagonists can initiate or lengthen REM sleep, whereas its lesion eliminates REM sleep (Friedman & Jones, 1984). A lesion around the locus coeruleus which inhibits muscle atonia leads to acting out of dreams in cats, i.e., lack of REM-associated paralysis (Morrison et al., 1983).

D) *a hypothalamic system* – hypocretin (orexin) neurons in the hypothalamus project their axons to the other sleep centres, i.e., basal forebrain, reticular formation, subcoeruleus and hypothalamic tuberomammillary nucleus actively coordinating whether the brain will be awake or asleep. Loss of orexin neurons leads to a medical condition called narcolepsy which leads to a sudden loss of muscle tone which resembles REM atonia while the person is fully awake, i.e., cataplexy.

2.4.2. A neurophysiological cycle of sleep and wakefulness

Acetylcholine produced by neurons in the upper pons activates the thalamus which sends a wakefulness-promoting signal to the cerebral cortex (Peplow, 2013, Figure 6 – Branch 1). Simultaneously, neurotransmitters including noradrenaline, serotonin, histamine and dopamine produced by pons, hypothalamus and other nearby regions prime the cerebral cortex to receive the signal from the thalamus (Peplow, 2013, Figure 6 – Branch 2). At the same time, to reinforce the arousal system, the hypothalamus releases hypocretin (orexin) (Peplow, 2013, Figure 6 – Branch 1).

Homeostatic drive increases sleep pressure linearly with the time of being awake via the accumulation of adenosine. Accumulation of adenosine triggers neuron activity in the ventrolateral preoptic nucleus which releases GABA and galanin. GABA and galanin attach to receptors in the hypothalamus and pons to inhibit the arousal systems (Peplow, 2013, Figure 6 – Sleep signal).

Along with the homeostatic control, the circadian system governed by Suprachiasmatic Nucleus (SCN) channels signals to the ventrolateral preoptic nucleus. SCN is activated by the sunlight received from the retina and inhibits the paraventricular nucleus located in the hypothalamus promoting wakefulness. Long axons of the paraventricular nucleus send a signal to the preganglionic sympathetic neurons in the spinal cord which in turn control the activity of the superior cervical ganglion which projects to the pineal gland responsible for melatonin releases. Melatonin is also called the "*Dracula hormone*" and the "*hormone of darkness*" because the pineal gland is inactive during the day and activates when the sun goes down starting the production of melatonin which is realised in the blood promoting sleep. Importantly, artificial light can suppress melatonin production even if the sun is down.

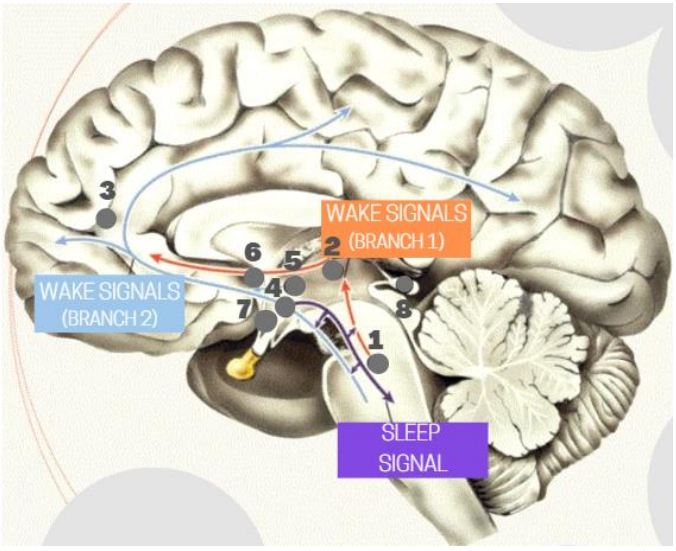


Figure 6. Anatomy of sleep. Sleep and wake are regulated by several neuronal systems and chemically distinct neuronal groups. Legend: 1. upper pons 2. thalamus 3. cerebral cortex 4. hypothalamus 5. lateral hypothalamus 6. ventrolateral preoptic nucleus 7. suprachiasmatic nucleus 8. pineal gland. The image was adapted from Peplow, 2013

2.5. Functions of sleep

Sleep is a naturally occurring state of a reversible loss of consciousness that puts the organism in a defenceless position in case of an eventual attack, which already suggests that the evolutionary benefits of sleep must significantly surpass potential risks. Increasing sleep debt and related unpleasant consequences of sleepiness mirrored by grogginess and being socially undesirable can be viewed as simple evolutionary tools to promote sleep (Siegel, J., 2009). In a nutshell, sleep plays role in stress response, regulation of appetite, endocrine, metabolic and immune regulation, mood, emotional, well-being, emotional stability, reward circuits, brain maturation, emotional maturation, regulation of pain, cognitive performance, memory consolidation and creativity (During & Kawai, 2017).

Total lack of sleep leads to death in mammals including humans (e.g. fatal familial insomnia[12]). Extensive sleep, however, is not a good sign either. A large meta-analytic study established a U-shaped association between the hours of sleep and all-cause mortality (Cappuccio et al., 2010). Both, short (commonly <7 h per night, often <5 h per night), and long sleep duration (commonly >8 or 9 h per night), were related to a greater risk of dying as much as 12% and 30%, respectively to individuals who slumber for 7 to 8 h per night in average (Cappuccio et al., 2010).

[12] *Fatal familial insomnia* is a very rare genetic disorder which is characterized by insomnia which worsen progressively over time causing hallucinations, delirium and death that comes on average in 18 months from the onset of symptoms (Schenkein, 2006).

2.5.1. Adaptive inactivity

The reduced metabolic activity during sleep, e.g., decreased muscle tension, lower heart and breathing rates, lower blood pressure and reduced core body temperature (especially during N2 and N3 sleep), suggest that *energy conservation* is one of the sleep functions (Breedlove & Watson, 2018). Further, as a brain spends more than 20% of the whole body's energy, it is hypothesised that sleep is necessary for performing cellular maintenance and preventing neuronal damage related to extensive activity during wakefulness (Vyazovskiy & Harris 2013).

2.5.2. Toxic proteins clearance

The activity of the glymphatic system is enhanced during sleep, especially SWS, which allows the brain the removal of toxins that build up during wakefulness (Xie et al., 2014). The inefficient functioning of the glymphatic system can contribute to the development and progression of neurodegenerative diseases such as Alzheimer's Disease which is characterized by aggregation of Aβ and tau tangles (Jessen et al., 2015). Importantly, sleep deprivation reduces clearance and promotes astrogliosis and network activity–driven tau and amyloid-beta release promoting the spread of AD pathology (Holth et al., 2018).

2.5.3. Synaptic homeostasis – is sleep a price that we pay for brain plasticity?

Sleep plays a key role in brain plasticity over the lifespan as well as maintenance of synaptic balance in the context of learning (Tononi&Cirelli 2003; Tononi&Cirelli 2005; Tononi&Cirelli 2014). According to the Synaptic Homeostasis Hypothesis, during wakefulness connection between neurones strengthen through the brain while during sleep the spontaneous activity renormalises net synaptic strength promoting the restoration of cellular homeostasis (Tononin and Cirelli, 2014). Due to the necessity to attend to an enormous number of stimuli and learning processes, during wakefulness, the brain is a slave of the present, therefore, sleep is a perfect time to conduct synaptic renormalization. Consequently, newly learned information can be integrated with already consolidated memories and the brain can start a new day refreshed and ready for the processing of new information.

2.5.4. Sleep-dependent memory consolidation

Sleep is necessary for memory consolidation and maintaining efficient cognitive function (Diekelmann & Born, 2010). Newly encoded memory traces are at first fragile and need to be strengthened to be consolidated long-term. Post-training sleep was shown to enhance hippocampal activity during the recall of word pairs 48h after learning which indicates hippocampal processing of declarative memories during sleep (Gais et al., 2007). The *Active System Memory Consolidation theory* states that sleep-dependent memory consolidation is coordinated by the interplay of hippocampal

ripples, thalamocortical spindles and neocortical slow oscillations (Born & Wilhelm 2012). During SWS, the hippocampus which plays a role in temporal memory storage replays the information encoded during the day. Then the reactivated memories pass through the thalamus allowing them to be incorporated into the neocortex for long-term storage. This long-term memory shift of hippocampal memories towards neocortex storage was shown to be disturbed by sleep deprivation following learning (Gais et al., 2007).

According to the *dual-process hypothesis* REM and NREM sleep are involved in different consolidation processes depending on the memory system (e.g., Gais & Born, 2004, Maquet, 2001). SWS facilitates consolidation of declarative, e.g., word-pair task, whereas REM of non-declarative memories, e.g., a serial reaction time motor skill task (e.g., Gais & Born, 2004, Maquet et al., 2000). It is hypothesised that learning during wakefulness induces synaptic changes locally which in turn induces local changes in slow-wave activity (SWA) that enhance newly encoded memories (Tononi&Cirelli 2014, Huber et al., 2004). A large number of studies demonstrated that learning before sleep influences post-learning sleep physiology on the macro and micro levels (e.g., Sanford et al., 2001, Ribeiro et al., 2004, Stickgold et al., 2000, Smith et al., 2004, Gais et al., 2002). For example, an increased density of rapid eye movements was observed during REM sleep following procedural encoding (Smith et al., 2004) and an increased density of SS following extensive training on verbal declarative memory (Gais et al., 2002).

Interestingly, incorporating elements of pre-sleep spatial learning into dreams was shown to be associated with enhanced memory consolidation and better virtual maze navigation task performance the next morning (Wamsley & Stickgold, 2019). The obtained results are consistent with the memory reactivation model of memory consolidation during sleep (Born & Wilhelm 2012) supporting the concept that cognitive activation of recent experiences during sleep is associated with subsequent, next-morning performance gains. The neuronal pattern of activity recorded during cognitive task performance (spatial environment encoding) was shown to be replayed during subsequent sleep (Peigneux et al., 2004). Furthermore, in mice, network-level offline reactivation of spatial memory traces during sleep predicts the future representational stability of place cells, i.e., long-term cognitive maps (Grosmark et al., 2021).

Good support for the rehearsal of newly acquired information during sleep is provided by memory reactivation tasks using cueing. Rash et al. (2007) have shown that cueing memories during N3 sleep improved visual memory performance. During the study, participants were asked to memorize the location of the objects on the screen while exposed to the smell of roses. During the subsequent sleep, the smell of roses (i.e., contextual cue) was presented during different sleep stages. It was shown that participants exposed to the rose scent during SWS demonstrated hippocampal response and

consequently recalled significantly more object locations compared to those who were exposed to the smell in other sleep stages or during wakefulness. Further, reactivating memories during sleep improves the performance of newly acquired complex motor skills (Chen et al., 2021). The study protocol included a myoelectric feedback task that required learning to control the myoelectric activity of particular arm muscles to move the cursor to reach 16 locations on the computer screen. Every localization was associated with a unique sound. After the training session, half of the sound stimuli were re-played during SWS aiming to reactivate memories of specific locations (i.e., auditory cueing). Strickling, during post-sleep task performance, the movements that were cued during sleep were executed faster and more smoothly in comparison to un-cued ones. The study shows great potential for the usage of targeted memory reactivation during sleep as a neurorehabilitation tool.

2.6. Consequences of sleep loss

Sleep deprivation is a way to explore the regulatory mechanisms of sleep and wake. Acute sleep deprivation refers to a period of continuous wakefulness exceeding 16 to 18h, whereas sleep restriction refers to too little sleep over a prolonged time (Banks et al., 2017). The most common behavioural changes related to prolonged wakefulness are irritability, problems with concentration and episodes of disorientation (Breedlove & Watson, 2018). Sleep debt can significantly impair cognitive performance which makes it a safety hazard as it can lead to workplace errors or traffic accidents caused by microsleep intrusions. Regardless of its harm, sleep restriction is a common characteristic of the 24/7 modern lifestyle. The sleep survey carried out by the Centres for Disease Control and Prevention has revealed that 35.2% of U.S. adults reported sleeping less than 7h per night, whereas more than two-thirds of U.S. high school students stated to sleep less than 8h during school week[13] (Centers for Disease Control and Prevention). Curiously, short sleep is commonly glorified in the public sphere, for example, the former British Prime Minister Margaret Thatcher said once "*Sleep is for wimps*", while another, modern member of the *sleepless club* - Donald J. Trump praised himself for being a short sleeper. Trump claimed that he needs only four hours of sleep a night which was questioned by an analysis of his controversial around-the-clock Twitter activity (Kryger, 2017).

2.6.1. Sleep loss and cognition

The first documented sleep deprivation study took place in the 19th century when the participants were awake from 36 to 90 hours (Patrick & Gilbert, 1896). This pioneering study revealed that memory and response time were severely impacted by prolonged wakefulness. Further, adults who underwent partial sleep deprivation, i.e., 6 or 4h of sleep per night for 2 weeks demonstrated

[13] Recommended sleep time for adolescence is 8 to 10 hours per night (Paruthi et al., 2016).

impairment in attention and reaction time tasks compared to controls sleeping 8h per night (Van Dongen et al., 2003). The cognitive deficit of the partially-sleep deprived participants was equal to 3 days and nights-long total sleep deprivation which indicates that the sleep debt and its neurobehavioral consequences accumulate over time (Van Dongen et al., 2003).

One of the most commonly used tasks to investigate the magnitude of sleep loss is Psychomotor Vigilance Task (PVT) which assesses vigilant attention (Dinges & Powell, 1985). Sleep loss leads to slower reaction time and an increased number of lapses (reaction time $\geq$ 500ms) which are believed to mirror microsleep intrusions (Lim & Dinges, 2008). While sleep debt accumulates, lapses are getting more frequent and longer which supports the *wake-state instability hypothesis* stating that sleep loss leads to moment-to-moment fluctuation between involuntary sleep initiation and maintenance of wakefulness (Doran et al., 2001, Chee et al., 2008). State instability leads to an elevated level of errors of omission (i.e., lapses) as well as errors of commission (responding in absence of stimuli) accompanied by timely normal responses (Doran et al., 2001). The effect of sleep loss was shown to be comparable to alcohol intoxication. The performance impairment after 17h of wakefulness was shown to be equivalent to having a mean blood alcohol concentration of 0.05% (Dawson & Reid, 1997).

Importantly, vulnerability to sleep loss varies significantly across individuals and is more consistent within individuals which implies that the response to sleep loss is *trait-like* (Van Dongen et al., 2004). This sleep-loss-trait-like response to sleep restriction is suggested to be attributed to genetic differences, such as a variable number of tandem repeat polymorphism in the clock gene PERIOD3 (PER3) and ADORA2A polymorphism (Rupp et al., 2013). Importantly, PER3 polymorphism was shown to be associated with differences in slow-wave sleep features showing the close relationship between homeostatic processes and the circadian clock in sleep regulation (Viola et al., 2007). The functional magnetic resonance imaging (fMRI) study showed that after one night of total sleep deprivation, vulnerable participants (i.e., individuals demonstrating worse cognitive performance in a visual selective attention task) showed a significant decline in frontotemporal activation during attentional lapses, whereas resilient participants demonstrated increased parietal activation (Chee et al., 2007). Further, drowsy participants (based on a video recording of eye activity during a scan) exhibited reduced resting cerebral blood flow activity in the frontoparietal region only after 1 night of 4-hours sleep restriction, which was not a case of non-drowsy individuals who showed increased cerebral blood flow in the basal forebrain and cingulate regions (Poudel et al., 2014). These findings suggest that some individuals might have certain compensatory mechanisms to handle sleep loss (i.e., non-drowsy participants showed enhanced activity in the arousal-promoting brain areas, whereas drowsy individuals were not able to maintain arousal in these brain regions) which can cause high interindividual differences in resilience towards sleep loss. Moreover, wake background EEG activity, especially in alpha and theta bands has

been related to the level of alertness, i.e., subjective sleepiness was negatively correlated with alpha power (across the scalp) and positively correlated with theta power (over the frontal areas) (Strijkstra et al., 2003).

Overall, total sleep deprivation decreases the speed of processing, attention (especially vigilant attention), constructive thinking, verbal memory, and spatial working memory and increases the formation of false memories (e.g., Banks et al., 2010, Dinges & Powell, 1985, Lim, & Dinges, 2008, Harrison & Horne, 1998, Heuer et al., 2005, Mullette-Gillman et al., 2015, Frenda et al., 2014, Banks et al., 2017). Curiously, reasoning and performance of complex cognitive tasks seem to be immune to the detrimental effect of acute sleep deprivation (Goel et al., 2009). From a psychological perspective, sleep deprivation impacts mood, decisions making in a social context, and emotional processing, decreases stress threshold and magnifies stress response (e.g., Grèzes et al., 2021, Zohar et al., 2005, Morales et al., 2019, Banks et al., 2017).

2.6.2. Sleep loss and health

Chronic short sleep impacts health, leading to an increased risk of common cold infections (Mohren et al., 2002), obesity, type 2 diabetes, hypertension, cardiovascular disease, Colorectal cancer, mood disorder, reduced quality of life and cognitive decline (Medic et al., 2017). Acute SD or sleep restriction was shown to affect the hormonal response, i.e., decreased insulin sensitivity, alert functioning of leptin and ghrelin which regulates appetite, and increased sympathetic response in healthy adults (Medic et al., 2017) as well as reduced antibody titers following vaccination (Lange et al., 2003).

2.7. Recovery sleep following sleep loss

The *recovery-sleep* characteristics depend on its duration, type of sleep deprivation, i.e., acute or chronic one and a recovery day following the sleep loss. It is suggested that a complete recovery from a period of sleep restriction requires more than 10 hours of recovery sleep or more than 3 days of 8 hours of sleep per night (Banks et al., 2017). On a sleep architecture level, sleep following sleep deprivation is associated with decreased sleep onset, longer total sleep duration and higher sleep efficiency (Caldwell & Caldwell, 1997, Caldwell & LeDuc, 1998, Beaumont et al., 2005, Bonnet & Rosa, 1987). Further, in response to the sleep loss, during the recovery night, the brain favours prolonged NREM sleep that is characterized by enhanced SWA (especially during the initial part of recovery sleep) with predominant activity in the frontal areas that are associated with higher cognitive functions and brain areas that have been very active during wakefulness (Dijk et al., 1990, Dijk, 2009, Huber et al.,

2004, Kattler et al., 1994, Vyazovskiy et al., 2004). REM sleep, on the other hand, shows its rebound during the second and further recovery nights following sleep loss (Beaumont et al., 2005).

Importantly, different cognitive functions recover at different rates. Following one night of total sleep deprivation, two nights of recovery sleep were shown to restore hippocampal connectivity to the baseline level but did *not* facilitate full recovery of memory performance in a scene recognition task (Chai et al., 2020). The study provides an interesting insight suggesting that recovered hippocampal functional connectivity is *not* sufficient to support efficient episodic memory performance. Generally, the more severe the sleep loss is, the longer the recovery sleep should be. In Lamond et al., 2007 study, participants were sleep-deprived for 1 or 2 nights, then one group had 5 consecutive nights of 6 h sleep opportunity and the other of 9 h. In both cases, i.e., 1 or 2 nights of sleep loss, the SWS returned to the baseline level after the first recovery night. Importantly, regardless of SWS rebound, in a more severe condition (i.e., two consecutive nights of total sleep deprivation), cognitive performance (PVT) was below the baseline following 5 nights of 9 hours of sleep (i.e., extended recovery sleep), whereas in the group sleep-deprived for one night, the performance returned to baseline after 9 hours of recovery sleep (Lamond et al., 2007).

2.8. The physiological regulation of sleep – two-process model

Following a distinction made by Borbély in 1982 (Borbély et al., 1982), sleep is regulated by two separate systems - circadian rhythm (Process C) and sleep-wake homeostasis (Process S) (see Figure 7). The two components of the sleep-wake regulatory system are neuroanatomically distinct but work in dynamic interaction in defining the timing, duration and quality of sleep and wakefulness. The investigation of the two systems separately is challenging and requires special experimental protocols such as ultra-short sleep/wake protocol, multiple nap protocol, forced desynchrony protocol, constant routine protocol, sleep restriction, total sleep deprivation or partial sleep deprivation protocols.

2.8.1. Sleep-wake homeostasis - Process S

Sleep-wake homeostasis (Process S) mirrors how sleep pressure accumulated during wakefulness and is released during sleep (Cirelli, 2009). The shape of Process S is a function of prior sleep and wakefulness. Process S generates the homeostatic sleep drive via a gradual increase of the hypnogenic chemical molecule - adenosine which is a by-product of adenosine triphosphate (ATP) metabolism. The concentration of extracellular adenosine leads to an increased desire to sleep (i.e., increased sleep pressure) and rises linearly with increased time awake. The longer one is awake, the more adenosine accumulates, i.e., the more built-up adenosine in the bloodstream, the sleepier one

becomes. The level of adenosine decreases following recovery sleep and can be silenced by caffeine which blocks adenosine receptors[14].

Prolonged wakefulness exceeding its physiological duration (~16 hours in humans) leads to increased sleepiness, worsens cognitive performance, and when sleep finally comes its duration and/or intensity are greater compared to the baseline conditions (Cirelli 2009). There is a strong correlation between the amount of slow-wave activity and sleep pressure, i.e., time of being awake (Dijk et al., 1990). For example, during the first recovery night following total sleep deprivation the brain will generate far more deep sleep, than light sleep or REM sleep which is known as SWS sleep '*rebound*' (Dijk, 2009). Importantly, slow-wave activity is considered an indicator of intensity and depth of sleep (Borbély et al., 1981). In rodents, this homeostatic sleep recovery was shown to disappear in case of chronic sleep loss and turning into the allostatic system[15] (Kim et al., 2007).

2.8.2. Circadian system - Process C

The circadian system (Process C) regulates the periodicity of the sleep-wake cycles within 24 hours and its main Zeitgeber (*time giver*) is sunlight (LeGates et al. 2014). The circadian system consolidates sleep during the dark phase and promotes wakefulness during the light phase. Circadian rhythmicity is governed by the SCN located in the anterior hypothalamus, situated directly above the optic chiasm. The retinohypothalamic pathway connects melanopsin-containing photosensitive retinal ganglion cells directly with SCN carrying information about light through the hypothalamus to entrain behaviours (Moore, 1982). Light information forwarded through the retinohypothalamic pathway travels to the SCN entraining the molecular clock to the light-dark cycles of life.

[14] Drinking coffee to overshadow sleepiness is like inviting a Trojan horse to the brain. Once caffeine stops being active, one will feel even stronger urge to sleep because sleep pressure kept increasing along with caffeine activity (half-life of caffeine takes on average five to seven hours) (Walker, 2017).

[15] *Allostasis* promotes maintaining homeostasis by implementation of the adaptive change to meet anticipated demands.

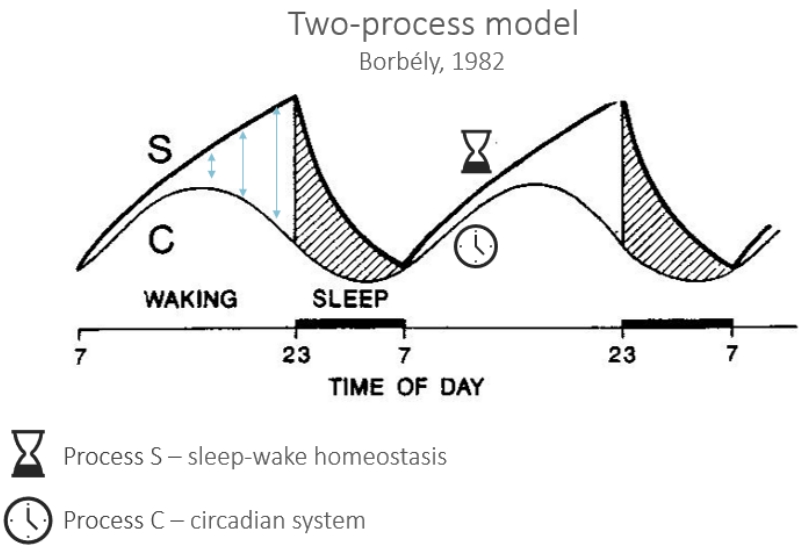


Figure 7. The two-process model of sleep regulation (Borbély et al., 1982). The model pictures the interaction between Process S and Process C, where Process S displays gradually raising sleep pressure over the daytime that drops sharply once a person falls asleep, while Process C is a constant sinusoid that cycles between day and night. The greater the distance between Process S and Process C, the greater the desire to sleep. The interaction between these two forces helps overcome the effect of increased sleep pressure (even in severe sleep deprivation scenarios) and keeps a person alert during the daytime (wakefulness-promoting zone). On the other hand, keeping a person awake after an extended time of wakefulness around 4 a.m. is challenging because of the sleep-promoting phase of Process C. The two-process model can be used to reliably predict alertness and neurocognitive functions in response to different sleep and wake scenarios (Van Dongen, 2004).

2.8.3. Suprachiasmatic nucleus - the body's master clock

One of the best support that SCN is a body's master clock was an experiment in golden hamsters that revealed that when SCN is lesioned and the animal is placed in constant dim light conditions, it expresses a completely random sleep-wake pattern that indicates loss of endogenous rhythmicity (Rusak, 1977). Further, transplantation of SCN in golden hamsters led to the adaptation of the circadian locomotor activity of the donor (Ralphs & Menaker, 1988).

The molecular clock is based on a transcriptional-translation negative feedback loop of core clock genes. Accordingly, cells in SCN produce two proteins called Clock and Bmal1 which by binding together promote the transcription of Period (Per1, Per2 and Per3) and Cryptochrome (Cry1 and Cry2) genes (Colwell, 2011). Once Per and Cry bind together, they inhibit the transcription of their own genes, however, once the connection between them is degraded, Clock-Bmal1 can promote transcription of Per and Cry again (i.e., a new, roughly 24h-long cycle is initiated again). The transcription for Per and Cry genes peaks during the period from the middle of the day until late in the day, while the PER and CRY proteins reach a peak in the early night (Colwell, 2011). Numerous cells contain this *molecular feedback loop* that facilitates the modulation of the circadian transcription of numerous genes (Colwell, 2011).

This 24-hour molecular cycle drives the 24-hours activity cycle of *each* SCN neuron giving the SCN "*time awareness*". Accordingly, the circadian system provides precise temporal instructions to the peripheral clocks located in various tissues throughout the body (e.g., liver, pancreas, digestive system, muscles) governing the temporal organization of numerous physiological processes starting from rest-activity patterns, and feeding-fasting cycle, through hormones secretion, glucose clearance, nutrition absorption, changes in the core body temperature, blood pressure, cardiac function, and gut motility, to bowel movement suppression, neuronal activity, plasticity and cognition (Richards & Gumz, 2012, Hartsock & Spencer, 2020, Xu et al., 2021).

Since humans are diurnal species, the SCN master clock is entrained by light received by the retina. SCN communicates the day (period of activity in presence of light) and night (period of sleep in absence of natural light) to the brain and other body organs coordinating central and peripheral clocks[16] via the hormone *melatonin* produced by the pineal gland. Secretion of melatonin to the bloodstream starts after dusk and rises successively until ~4 AM, afterwards, it begins to drop along with dawn (Walker, 2017). Accordingly, the rising level of melatonin informs the body about approaching sleep time and its decrease tells the body that the night is over, and it is time to wake up. During the day, the level of melatonin is almost undetectably low. Besides melatonin, plasma cortisol levels, as well as core body temperature, show characteristic circadian fluctuations (Walker, 2017). If we assume that the time of sleep is between 11 p.m. and 7 a.m. plasma cortisol starts rising in the early morning around ~3 a.m. and decreases sharply after 2-3h upon awakening, whereas body temperature peaks in the evening and decreases throughout the night with the lowest point in the early morning hours after which it starts raising again.

2.8.4. Circadian rhythmicity and cognitive performance

Waking behaviour and most neurobehavioral functions are modulated by the circadian clock. The temporal profile of alertness, (an ability to sustain attention and wake neurobehavioral functioning) reflects the interaction of the circadian process with sleep homeostasis processes (Gabehart & Van Dongen, 2017). Investigating the modulation of neurobehavioral functions by the circadian clock allows for predicting the occurrence of cognitive performance deficits throughout the circadian cycle (Gabehart & Van Dongen, 2017).

The circadian rhythmicity of cognitive performance mirrors functional changes in the electrical brain activity over time of day. Diurnal changes were shown in the amplitude of event-related potentials

[16] Several tissues and organs are able to generate circadian rhythms in vivo, in SCN-independent manner. Nevertheless, SCN remains the commander of the army of the mammalian circadian system (Turek & Zee, 2017).

(Münte et al., 1987, Stolz et al., 1988) and in hemispheric differences (Corbera et al., 1993) that suggest distinct circadian rhythms for the left and right hemispheres (Gabehart & Van Dongen, 2017). Circadian changes have been also described in slow-wave activity and were closely linked to waking brain functions (Dijk & Czeisler, 1995, Lazar et al., 2015). Further, the regional modulation of brain circadian rhythmicity was shown by the fMRI study that assessed brain activity during repeated PVT during baseline wakefulness, total SD (42h of wakefulness) and after the recovery night (Muto et al., 2016). The study included 13 fMRI imagining sessions that allowed exceptional temporal resolution across the circadian cycle. Circadian modulation of subcortical areas followed the melatonin rhythmicity profile. These subcortical activity fluctuations were not influenced by sleep pressure. Other cortical areas showed significant circadian rhythmicity and their phase varied across the different brain regions and was impacted by sleep debt. These cortical activities decreased significantly with accumulated homeostatic sleep pressure.

Furthermore, sustained attention (e.g., a continuous performance task), working memory (e.g., spatial n-back task) and cognitive inhibition (e.g., go-no-go task) demonstrate a high level of stability in performance during the daytime, whereas low stability during night-time and early morning (Valdez et al., 2010, Groeger et al., 2008, Harrison et al, 2007). Performance on the Stroop task with shifting criteria assessing cognitive inhibition was shown to be worse during early morning hours (i.e., between 3 AM-6 AM) (García et al., 2011). Also, declarative memory performance (memorization of prose passages) demonstrates time-of-a-day fluctuation with performance being the best during daytime and the worse at circadian night (Johnson et al., 1992).

Inter-individual circadian preferences[17] (that change across the lifespan) modulate cognitive performance via differences in the phase of the endogenous circadian rhythmicity (associated with core body temperature and melatonin profile) that can result in different times of peak performance (Kerkhof & Van Dongen, 1996). Notably, a significant difference in memory performance between young and older adults was shown only when older participants were tested in the evening, i.e., at the not optimal time of the day for elderly who show predominantly advanced circadian patterns with peak cognitive performance in the morning hours (Hasher et al., 2002). Not acknowledging these circadian preferences can exaggerate the age-attributed differences in cognitive performance (Hasher et al., 2005). Overall, time of day modulates cognitive performance on numerous cognitive tasks, moreover,

[17] *Chronotype* is a behavioural display of underlying circadian rhythms. By using, for instance, the Morningness-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1976) assessing circadian preference, we can distinguish, intermediate, morning- and evening-type chronotype types. Accordingly, some individuals are naturally more alert and perform better in the morning (morning larks) and the others are at their best towards evening hours (night owls).

these performance fluctuations depend also on inter-individual circadian preferences (Schmidt et al., 2007).

Besides circadian modulation, wake performance can be influenced by other factors such as physical activity, social engagement, body posture, sensory stimulation or caffeine that can mask[18] circadian rhythmicity. Assessment of circadian rhythmicity in neurobehavioral functioning can be also contaminated by the *compensatory effect* when the participant tries to keep pace with the demands of a task by investing more effort (it is especially notable when the participants receive the feedback), *practice effect* (i.e., learning curve) and *task aptitude* which might show high inter-individual variability (Gabehart & Van Dongen, 2017). Some individuals experience as well mid-afternoon hours *afternoon dip* phenomenon (also called post-lunch dip) which is visible in the profile of decreased core body temperature and poorer neurocognitive functioning (Monk, 2005).

2.8.5. Circadian rhythmicity versus sleep homeostasis – experimental protocols

Interaction of sleep homeostasis and circadian rhythmicity drive waking neurobehavioral functioning. The wakefulness-promoting zone safeguards cognitive performance to avert neurobehavioral impairment related to accumulated sleep pressure allowing to maintain relatively stable cognitive performance throughout the day. However, in sleep deprivation protocols, alertness and neurobehavioral functioning are steadily impaired by increasing sleep loss. Consequently, the circadian pattern of cognitive performance is overshadowed by successively accumulating sleep pressure. The moment-to-moment *instability* that is characteristic of sleep deprivation is reflected by, for example, an increased number of attentional lapses on the PVT and relying on compensatory mechanisms (indicated as *normal* RT) such as putting extra effort to perform the task[19] while fighting sleepiness (Gabehart & Van Dongen, 2017).

Process C and Process S interact to promote sleepiness and wakefulness. The constant routine protocol, where the participant stays awake for at least 24h in a fixed body position in a highly controlled environment (i.e., constant room temperature and light intensity, standardized meals) is a golden standard for measuring circadian rhythmicity (i.e., circadian phase and amplitude). How can we measure Process C and Process S separately? One of the interventions measuring Process S and Process C is called *forced*[20] *desynchrony* protocol. To illustrate the example, Zhou et al., 2011 used the forced desynchrony protocol, where the participants followed a 28h day repeated in 7 cycles, spending 9.33h in bed and

[18] *Masking* describes a situation where external noncircadian factor influences measurement of circadian rhythmicity, e.g., drinking Coca-Cola right before reaction time assessment during sleep deprivation protocol.
[19] Mental effort can be assessed using subjective mental effort task using Visual Analogue Scale, where the participant is asked to indicate how much of mental effort and/or how cognitively demanding was given task.
[20] It is called *forced* because the internal body clock cannot synchronize with prolonged day imposed by protocol.

18.67h out of bed in dim light conditions. A sleep duration of 9.33h in 28h days gives around 8h of sleep every 24h which is a sufficient amount to avoid accumulating sleep pressure across the protocol (associated with Process S). Importantly, 28h days is too long for a human to entrain and the participants run on a roughly 24h endogenous schedule (indicated by core body temperature and alertness levels). In the described study, the second group went under sleep-restricted conditions, where the participants slept for 4.67h and were out of bed for 23.33h. The performance on the PVT task declined with time awake for both groups and was worse in the sleep-restricted group (i.e., less sleep time over 24h). Importantly, the reaction time was changing across the circadian phase, i.e., changes in performance reflected Process C. On the other hand, Process S was mirrored in the change in performance between standard sleep conditions and restricted sleep conditions.

Another commonly used protocol is the napping protocol (multi-nap protocol), where sleep pressure (Process S) is eliminated so Process C is reflected, i.e., frequent naps elevate sleep pressure, so Process S remains rather constant. These experimental protocols show nicely wake maintenance zone mirroring peak daily alertness rhythms and sleep-promoting zones, where alertness drops (as the circadian oscillator promotes sleep). Circadian researchers commonly implement a between-subject design with random assignment to either sleep deprivation protocol (SD) or nap protocol (Graw et al., 2004, Reichert et al., 2017, Cajochen et al., 2001, Maire et al., 2018, Sagaspe et al., 2012, Birchler-Pedross et al.,2009, Blatter et al.,2005). In the SD protocol, the participant stays awake for a prolonged period, usually around 40h, whereas in the nap protocol, the individual receives multiple short sleep opportunities, i.e., naps at regular intervals of time (usually 9-10 per 40h-long protocol) (Figure 8). Appendix 1 - *Circadian rhythmicity versus sleep homeostasis experimental protocols - summary table* provides an overview of studies that implemented SD and nap protocol protocols.

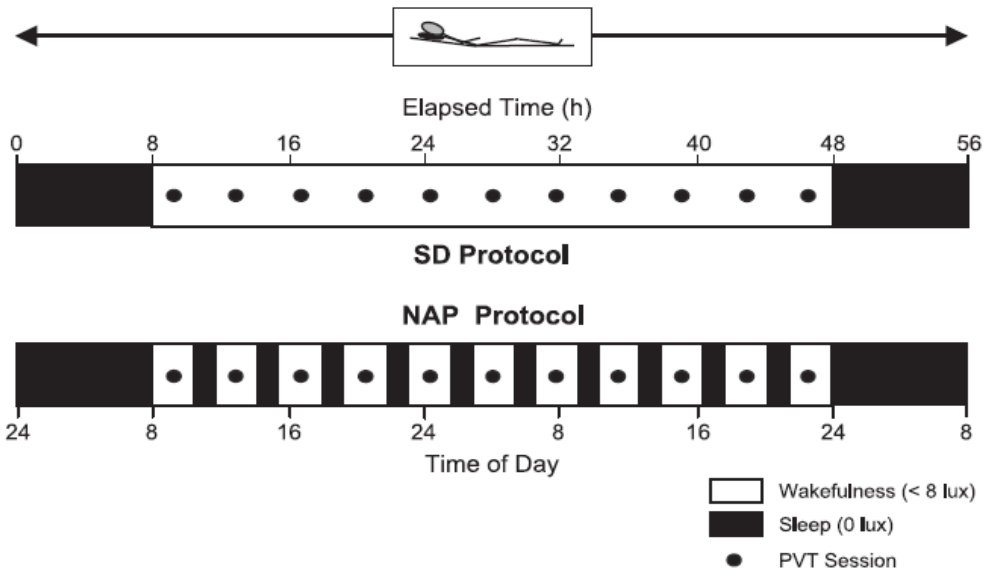


Figure 8. An example of circadian study design investigates to what extent circadian timing and the homeostatic sleep drive influence vigilant attention performance (Graw et al., 2004). The study consisted of two 40-h constant posture protocols in a balanced crossover design with high sleep pressure conditions (sleep deprivation protocol), where the participant undergoes total sleep deprivation (40h) and low sleep pressure conditions (nap protocol), where each 150 min of wakefulness is followed by 75 min of sleep (10 naps in total). The dots indicate the time of Psychomotor Vigilance Task (PVT) assessments which were scheduled precisely every 225 min starting from 75 min after lights were on, i.e., the end of baseline night (black block at the beginning of protocols). In the nap protocol, the PVT was scheduled 75 min before each nap. Figure Graw et al., 2004

2.9. Sleep and ageing

The integrity of sleep in older age is affected by changes in circadian and homeostatic processes, age-related comorbidities, and taking medication. Compromised sleep in older people can mirror physiological ageing at the systems, cellular and molecular levels (Bliwise & Scullin, 2017). For instance, subjective poor sleep in a cohort of middle-aged and older adults was associated with telomere shortening which is a biomarker of ageing (Cribbet et al., 2014). Interestingly, the self-reported short and long duration of sleep in middle-aged adults at the baseline were associated with cognitive decline during a follow-up 28 years later (Scullin & Bliwise, 2015), which supports nicely the U-shaped association between sleep and health described in the review paper by Cappuccio et al., 2010.

One of the hallmarks of ageing in sleep architecture is a reduction of SWS. It was reported that the medial-frontal cortex regions responsible for generating SWS show the most prominent age-related grey-matter atrophy (Mander et al., 2013). The severity of atrophy was associated with a more prominent loss of deep sleep (SWS). Elderly participants exhibited a 70% reduction in deep sleep in comparison to young adults. Moreover, older adults who generated less SWS had significantly worse performance on verbal learning tasks assessed by the paired-associate learning task. The selective atrophy within the medial-frontal cortex predicted impaired hippocampal memory performance which effect was mediated by a decrease in SWS. Furthermore, the temporal impairment of slow wave-spindle

coupling leads to diminished overnight memory consolidation and accelerated forgetting (Helfrich et al., 2017). These results suggest that the reduced amount of SWS in elderly individuals can be responsible for age-related memory complaints. Importantly, SWS activity can be partially attributed to the variable number of tandem repeats in PER3 polymorphism which was shown to partially influence the amount of delta activity (that is characteristic of SWS) (Viola et al., 2012). This genetic polymorphism might partially explain significant inter-individual variability in sleep in older age.

Further, microarchitecture features like sleep spindle density and K-complexes decrease with age. An amplitude of auditory evoked K-complexes, as well as the ability to elicit KC, is significantly reduced in older relative to younger adults (Colrain et al., 2010). Middle-aged and elderly adults have also a reduced number, amplitude and duration of SS, particularly in anterior channels (Martin et al., 2013). These spindles alterations can in turn affect memory consolidation and sleep protective mechanisms.

Older adults experience also a modest reduction in REM sleep duration (0.6% per decade) that emerges much later than age-related NREM sleep alterations (e.g., Carrier et al., 2001; Floyd et al., 2007; Scarpelli et al., 2019, Mander, 2020). Qualitative differences in REM sleep present in older adults (even if REM duration reduction is minimal) include increased awakenings from REM sleep, decreased REM latency, decreased REM density, and shorter and more disorganized REM bursts, particularly in adults over 65 years (e.g., Vegni et al., 2001; Darchia et al., 2003; Conte et al., 2014, Mander, 2020). Further, it was shown that older adults demonstrated changes in REM sleep microarchitecture, i.e., spectral power reduction across the delta, theta, and alpha frequencies particularly over central derivations (Landolt et al., 1996, Scarpelli et al., 2019, Mander, 2020). It remains unknown why REM sleep microarchitecture is reduced in ageing, and whether these changes are functionally relevant or epiphenomenal. Importantly, the decline in REM quantity was associated with worsened cognitive functions which might suggest that a decline in REM sleep leads to cognitive impairment or both, REM sleep and cognition are diminished by weakened cholinergic neurotransmission (Scullin &. Bliwise, 2015).

From the neurobiological perspective, the number of sleep-promoting neurons located in the VPN of the hypothalamus is progressively decreasing with age, which can contribute to fragmented sleep (Lim et al., 2014). This is in line with the results of the Sleep Heart Health Study that followed participants in the age range from 37 to 92 years and showed that the *arousal index* was increasing linearly with advancing age (Redline et al., 2004). The number of brief arousals is an important feature of sleep microarchitecture as it is a good estimate of sleep fragmentation. Sleep fragmentation in older adults was shown to be associated with accelerated ageing and activation of microglia as well as worse

cognitive performance which suggests that microglia ageing can partially contribute to cognitive impairment (Kaneshwaran et al., 2019). Moreover, sleep fragmentation in the elderly predicts also the risk of falls (Stone et al., 2008) and is commonly linked with nocturia[21].

Sleep disorders are common in older age, for example, insomnia symptoms affect 50% of adults aged 65 years and above (Ohayon et al., 2002). The prevalence of obstructive sleep apnoea in elderly adults is estimated to be 70% in men and 56% in women (Miner et al., 2017), whereas the percentage of Restless Leg Syndrome in older adults ranges from 9-20% (Hornyak & Trenkwalder, 2004). Also, the incidence of REM sleep Behavior disorder which is a prodromal phase of Parkinson's Disease increases with age (Tekriwal et al., 2016).

Further, it is hypothesised that daily naps, commonly seen in older adults are a visible manifestation of sleep fragmentation. Interestingly, when the short time of nocturnal sleep was accounted for, daytime naps were protective, while the opposite holds for long night slumber exceeding 9 hours where naps were associated with greater mortality risk (Cohen-Mansfield & Perach, 2012). Nevertheless, there is no agreement that napping[22] in older life is harmful or beneficial (Zhang et al., 2020). A recent review paper stated that the discrepancy in reported napping in older adults across different studies ranges from 20 to 60% (Zhang et al., 2020). What is consistent across the study is that elderly individuals take more naps than younger age groups.

2.10. Circadian rhythms and ageing

Altered circadian rhythms mirrored by decreased circadian amplitude, fragmentation and weakening of rest-wake cycles, as well as, reduced sensitivity to entraining signals (e.g., sunlight, social interactions) are common in ageing (Tranah et al., 2017). Considerably, it is not clear if the deterioration in circadian rhythmicity affects health directly or is rather an indicator of other age-related health problems.

Elderly adults present usually an *advanced* phase sleep pattern, meaning that they have earlier bedtime and wake-up times as well as an earlier daily alertness peak compared to younger individuals which is related to the advanced timing of melatonin peak (Czeisler et al., 1992). It is not clear what exactly causes these age-related changes in circadian rhythmicity. It was suggested that alteration in circadian rhythms can be partially attributed to age-related SCN changes (Hofman & Swaab, 2006).

[21] The night-time need for urination, i.e., *nocturia* might lead to the situation where an individual feels foggy upon awakening and then the change from the recumbent position to stand may cause blood racing from the head down towards the legs what in turn can lead to light-headedness and feeling unsteady on feet what can consequently lead to a fall (Walker, 2018). The injuries related to potential falls can in turn exacerbate sleep, for example, due to Traumatic Brain Injury (TBI) (Stewart et al., 2022).

[22] Notably, "*a nap*" does not have a well-established definition in scientific jargon. Additionally, it should be also taken into consideration that napping behaviour might have also cultural roots, e.g., siesta.

Animal models showed that advanced age leads to molecular changes such as altered expression of clock genes and abnormal neurochemistry of SCN (Hofman & Swaab, 2006). Moreover, the peripheral circadian clocks show age-related changes mirrored by arrhythmic activity (Yamazaki et al., 2002).

Age-attributed visual system losses (e.g., cataracts) can contribute to impaired circadian photoreception and thus to impaired circadian entrainment to light. Consequently, elderly people with visual impairments were shown to be 30% to 60% more likely to have impaired night-time sleep compared to controls without visual complaints (Asplund et al., 2000). Additionally, elderly people often spend less time in bright light which might be related to age-related mobility issues or poor physical health. A potentially beneficial way to shift the phase of circadian rhythmicity, as well as improve cognition is the bright light stimulation intervention (Rubiño et al., 2020). Physical activity might also help in regulating circadian activity patterns. Importantly, circadian disturbances are a common feature of depression which might by itself lead to less stable and fragmented rest-activity patterns.

2.11. Sleep in Alzheimer's disease

Sleep alterations worsen with progressing AD neurodegeneration which shows a significant bidirectional relationship between advancing brain atrophy, cognitive impairment and disrupted sleep (Ju et al., 2013, Bubu et al., 2017). Importantly, sleep disturbances can worsen the quality of life of patients and caregivers and may lead to premature institutionalization[23] (Petit et al., 2017). Sleep alterations occur in up to 25% of mild to moderate and in about 50% of moderate to severe AD patients with insomnia, a high index of nocturnal awakenings, early morning awakenings and daytime sleepiness being the most common complaints (Petit et al., 2017). Sleep alterations can lead to functional outcomes, for example, the degree of daytime sleepiness measured by the Multiple Latency test[24] was associated with the degree of cognitive dysfunction in mild and moderate AD patients (Bonanni et al., 2005). Furthermore, obstructive sleep apnoea which leads to prominent sleep fragmentation, shallower sleep and daytime sleepiness is highly prevalent among AD patients. According to the meta-analysis, AD patients have a five times higher chance of being diagnosed with obstructive sleep apnoea compared to cognitively intact individuals of similar age (Emamian et al., 2016).

Individuals reporting short sleep (≤ 6h) at ages 50, 60 and 70 had a 30% increased risk of late-onset dementia compared to those having a normal sleep duration (7h) (Sabia et al., 2021). The effect was independent of sociodemographic, behavioural, cardiometabolic, and mental health outcomes. Interestingly, another, recent study in older adults showed a U-shaped association between cognitive

[23] Sleep problems were identified as the major cause of early institutionalization in dementia patients (Petit et al., 2017).
[24] In the *multiple sleep latency test* an individual receives 4-5 diurnal nap opportunities, every two hours, two hours each. The test is used to measure daytime sleepiness that is based on person's sleep latency to N1 and latency to REM during the naps.

decline (assessed by a preclinical Alzheimer's cognitive composite score) and total sleep time (TST), sleep efficiency (SE), time in NREM and REM sleep and slow-wave activity (Lucey et al., 2021). The effects were adjusted for age, tau/Aβ ratio, biological sex, years of education and APOE-ε4 carriership status. Either short (> 4.5h per night) or long sleep (< 6.5h per night) was associated with more pronounced cognitive decline in comparison to individuals whose sleep had moderate duration. In the study, 100 participants underwent several cognitive assessments over 4.5 years and 4-6 sleep assessments. At the time of the study, 1 participant had MCI, 11 individuals had a subtle MCI and the rest were cognitively intact. The study implies that sleep *quality* plays a more important role in cognitive decline than total sleep *duration* as stable cognitive functioning over time was associated with a middle range of TST, time in NREM and REM and SWA (<1Hz).

2.11.1. Polysomnographic changes in AD

Sleep architecture in AD patients resembles super-accelerated-ageing and pathological changes can be seen in all sleep stages (Table 2). Accordingly, polysomnography findings in AD patients showed prominent sleep fragmentation (reflected by a high number of prolonged arousals), increased percentage of N1 and reduced percentage of SWS (Petit et al., 2017, Prinz et al., 1982). Importantly, AD neuropathology leads to the accumulation of neurofibrillary tangles, neuritic plaques and neuronal loss which supports the idea that insufficient functioning of the brain's glymphatic system during SWS can play an important role in AD pathogenesis (Xie et al., 2014). There are also AD-specific changes in sleep architecture which are not common in ageing. For example, the percentage of REM sleep which is stable throughout adulthood was shown to be reduced in AD patients (Montplaisir et al., 1995). Moreover, REM sleep EEG slowing for left frontal, left and right parieto-occipital and left temporal regions correlated positively with the Mini-Mental State Examination score in AD patients (Montplaisir et al., 1996). The reduced percentage of REM sleep and longer REM latency were also associated with a higher risk of dementia (Pase et al., 2017).

Neurodegenerative changes can alter sleep architecture to the extent that sleep EEG looks nearly featureless. For example, sleep spindles and K-complexes show lower amplitude, shorter duration and lower density in comparison to healthy age-matched controls (Montplaisir et al., 1995, Ktonas et al., 2007, Gorgoni et al., 2016, Gennaro et al. 2017). Further, elicited K-complexes in AD patients were shown to be less frequent and lower in amplitude in response to auditory stimulation (Crowley et al., 2005). The ability to produce K-complexes was associated negatively with the severity of AD which suggests that neurodegenerative changes may impair the ability to generate high amplitude features typical for NREM sleep making the AD polysomnographic recording more featureless with advancing AD pathology. That in turn makes the differentiation between stages N1 and N2 troublesome,

moreover, the percentage of indeterminate NREM sleep is further increased by the loss of high voltage delta waves that are hallmarks of SWS (Petit et al., 2017). These changes in sleep architecture seen in AD patients make sleep staging very challenging.

Authors	Subjects (N/age/sex/)	Outcome measures	main findings
De Gennaro et al. 2017	n=20 AD patients *sex(men):* 7 *age:* 72.0 n= 20 HC *sex(men):*12 *age:* 70.3 yrs.	- a one night of PSG - clinical characteristics (MMSE, HDRS, STAI Y-1, STAI Y-2 and PSQI scores)	**A significant 40% decrease of frontal, spontaneous KC density during stage N2 in AD patients in comparison to healthy controls.** KC density was correlated with MMSE scores ($p \leq .0001$). The KC density allowed to distinguish between AD and HCs in 80% of cases.
Reda et al., 2016	n= 20 amnesic MCI patients *sex(men):* 8M age: 72.20 yrs. n= 20 AD patients *sex(men):* 12 age: 70.3 yrs. n= 20 HC *sex(men):* 12 age: 70.3 yrs.	- a one night of PSG - clinical characteristics (MMSE, HDRS, STAI Y-1, STAI Y-2 and PSQI scores)	**An alteration of KC density in N2 seems to be related to the only symptomatic phase of AD.** A sig. difference in KC density was found between AD and MCI patients ($p \leq .05$), as well as AD and HCs ($p \leq .05$), however, there was no sig. difference between MCI and HCs ($p \leq .87$) A sig. negative correlation was shown ($r = 0.38$, $p = .003$) between MMSE scores and KC density.
Gorgoni et al., 2016	n= 15 AD patients *sex(men):* 5 *age:* 70.80 n= 15 amnesic MCI patients *sex(men):* 6 *age:* 71.10 n = 15 HC *sex(men):* 10 *age:* 70.80	- a one night of PSG - clinical characteristics (MMSE, HDRS, STAI Y-1, STAI Y-2 and PSQI scores)	**Fast spindles (13–15Hz) showed a significant reduction in AD and MCI patients as compared to healthy controls ($p \leq 0.05$).** Sleep spindles density was negatively correlated with MMSE scores ($p \leq 0.0001$).
Pase et al., 2017	n= 32 cases of dementia (including 24 of AD) *sex(men):* 11 *age:* 69	- home-based PSG - cognitive screening (MMSE)	**Reduced percentage of REM sleep and longer REM sleep latency** (i.e., the time between falling asleep and researching REM sleep) were both associated with a higher risk of incident dementia, i.e., each percentage reduction in REM sleep was related to approx. a 9% increase in the risk of dementia (hazard ratio 0.91; 95% CI 0.86, 0.97). The interaction APOE-ε4 allele status x REM sleep percentage was found ($p = .05$).
Musiek, et al., 2018	n=189 of cognitively normal adults *sex(men):* 68 *age:* 66.6 years 74% (139) of subjects were amyloid negative	- actigraphy - preclinical AD was assessed by longitudinal clinical assessment, amyloid imaging with Pittsburgh Compound B, and CSF biomarker collection.	**Preclinical AD is associated with rest-activity rhythm fragmentation.** Escalating pTau to Aβ42 ratio (indicating more AD pathology) was associated with increasing circadian fragmentation as measured by intradaily variability ($\beta = .231$; $p = .008$). No correlation between SE and any circadian variables was found. Circadian fragmentation did not correlate with any night sleep variables, however, there was a significant correlation between the number of naps and circadian fragmentation ($r = 0.152$; $p = .04$).

Table 2. Chosen publications showing alterations in sleep architecture and rest-activity patterns in AD. Abbreviations: *CSF* – cerebrospinal fluid; *HCs* – Healthy Controls; *HDRS* - Hamilton Depression Rating Scale; *KC* - K-Complex; *MMSE* - Mini-Mental State Examination; *PSQI* - Pittsburgh Sleep Quality Index; *STAI Y-1* - State-Trait Anxiety Index Form 1; *STAI Y-2* - State-Trait Anxiety Index Form 2

2.12. Circadian dysregulation in Alzheimer's disease

Sleep-wake alterations are a common characteristic of AD and the magnitude of the decline in the amplitude of the circadian rest-activity rhythm was shown to be associated with AD severity (Witting et al.,1990). The degree of AD pathology was correlated with reduced melatonin secretion (Mishima et al., 1999) and it was suggested that melatonin dysfunction can be caused by impaired sympathetic regulation of pineal melatonin biosynthesis by the SCN (Wu & Swaab, 2005) which is modulated by the nucleus basalis of Meynert which affected in the earliest stages of AD-neurodegeneration (Petit et al., 2017).

Actigraphy studies in AD patients have shown increased fragmentation of sleep-wake rhythmicity manifested by decreased activity, more nocturnal wake time, more daytime napping, reduced amplitude and phase delay of circadian variability in core body temperature (Carvalho-Bos, et al., 2007, van Someren et al., 1996). Circadian rest-activity rhythm fragmentation was shown to be associated with AD neurodegeneration (CSF pTau to Aβ42 ratio) (Musiek, et al., 2018). Further, higher Interdaily Instability was associated with worse cognitive impairment and depression in women with dementia (Carvalho-Bos et al., 2007). Importantly, lesser exposure to synchronizing Zeitgabers related to age-related impairment to light entrainment, less time spent in bright light, reduced physical activities and less structured social life can further worsen circadian alterations in AD patients (Abbott et al., 2017).

2.13. Impact of APOE genotype on nonrespiratory sleep parameters in Alzheimer's patients

Research on the association between APOE polymorphism and nonrespiratory sleep parameters in MCI and AD patients provided contradictory results. In one study, sleep disorders, irregular sleep-wake patterns and insomnia were shown to be more frequently in AD patients who are APOE-3/4 carriers (Cacabelos et al., 1996), while Yesavage et al., 2004 reported that greater deterioration of sleep parameters in the ε4 non-carriers. No link between APOE genotype and sleep satisfaction in AD patients was found (de Oliveria et al., 2014), further APOE polymorphism was shown to not influence the development of sleep disturbances in AD patients (Craig et al., 2006).

A study following AD patients from the early stages of the disease reported that in the case of ε4 carriers, Wake After Sleep Onset (WASO) was associated with poorer cognitive functions (Yesavage et al., 2004). However, for non-ε4 carriers, several sleep parameters, i.e., WASO, TST, SE and the amplitude of rest-activity rhythm were also associated with worsened cognitive decline (Mini-Mental State Examination) over disease progression. Counterintuitively, the study suggests a more severe impairment of sleep parameters in ε4 non-carriers. Another research suggested that the effect of ε4 on sleep alterations depends on gender and severity of AD load (Koo et al., 2019). Overall, sleep

disturbances were more frequent in males (42.4%) than in females (35.1%), and in ε4 carriers (42.3%) compared to ε4-non-carriers (36.5%) (n=2,368). Notably, in individuals demonstrating no to low AD pathology, female ε4 carriers had significantly more severe sleep alterations compared to non-carriers.

A polysomnographic study on healthy elderly and MCI patients revealed a significant shortening of REM sleep which was more prominent in ε4 carriers, along with increased fragmentations of SWS in MCI patients compared to healthy controls (Hita-Yañez et al., 2012). Demonstrated sleep alterations were not associated with memory performance in MCI patients, yet boosted REM sleep was related to improved immediate recall in MCI ε4 non-carriers. The following study of the same group reported that neither APOE-ε4 polymorphism nor self-reported sleep and daytime sleepiness predicted the amount of REM sleep in healthy participants and patients with MCI (Hita-Yañez et al., 2013). Overall, it is plausible that physiologically-driven sleep parameters are more sensitive to the influence of the ε4 allele compared to self-assessed sleep.

Interestingly, administration of anticholinergic medications was correlated with poorer sleep quality, impaired cognitive performance and mood only in elderly ε4 allele carriers compared to non-carriers (Nebes et al., 2013) suggesting that sleep of elderly ε4 carriers is more vulnerable to perturbations. Moreover, sleep disturbance assessed by component K of the neuropsychiatric inventory was significantly associated with ε4 heterozygosity in the participants without clinical dementia and with ε4 homozygosity in the cognitively impaired group. The study support hypothesis that APOE-ε4 carriership affects sleep by mechanisms independent of the extent of AD neuropathological change (Blackman et al., 2022).

Further, it was shown that AD APOE-ε4 homozygotes had lower melatonin levels (indicating dysregulation of circadian rhythms) compared to APOE-ε3/ε4 carriers (Liu et al., 1999). Importantly, the secretion of melatonin was shown to be associated with the severity of sleep disturbance in AD patients (Mishima et al., 1999). It is also possible that APOE polymorphism can influence, for example, the rate of aggregation of Tau and/or Aβ in the circadian master clock - suprachiasmatic nucleus and subsequently alters circadian rhythmicity in AD pathology.

2.14. Impact of APOE polymorphism on sleep in cognitively intact adults

Surprisingly there are not many publications focusing on the associations between the ε4 allele and nonrespiratory sleep parameters in cognitively intact adults. Nevertheless, APOE polymorphism was shown to influence sleep, circadian rhythmicity and moderate accumulation of proteins linked to AD (Asada et al., 2000, Burke et al., 2016, Camargos, et al., 2019, Drogos et al., 2016, Hwang et al., 2018, Ju et al., 2013, Kahya, M., et al., 2017, Lim et al., 2013, López-García et al., 2021, Lysen et al., 2020, Muto et al., 2020, Sapira et al., 2016, Spira et al., 2017, Tranah et al., 2018, Tsapanou et al., 2019). The sub-

chapter summarizes studies investigating the impact of the APOE genotype as a non-modifiable risk factor of AD on non-respiratory sleep parameters in non-demented adults. All the papers considering APOE status in relationship with sleep and activity-wake patterns were included even if APOE was only a secondary analysis, not related to the main scope of the publication (e.g., Muto et al., 2020). A table with a summary of the discussed study can be found in Appendix 2 - *Impact of APOE polymorphism on sleep in cognitively intact adults – summary table*.

2.14.1. Self-reported sleep and APOE

Self-reported sleep duration was shown to be influenced by the presence of the APOE-ε4 allele with APOE-ε4/ε4 carriers having greater odds of reporting a shorter sleep duration (<7h) compared to non-carriers and ε3/ε4 carriers (Spira et al., 2016). Further, the APOE genotype seems to modulate vulnerability to sleep disturbances. Cognitively intact elderly ε4 homozygotes were shown to have 50% greater odds of experiencing problems with falling or staying asleep relative to ε4 heterozygotes and ε3/ε3 carriers (Spira et al., 2017). Interestingly, a recent study revealed that insomnia and grey matter volume are modulated by APOE-ε4 status (Grau-Rivera et al., 2020). The presence of insomnia in APOE-ε4 carriers was associated with lower grey matter volumes, whereas absence with higher volumes in several regions, including the left angular gyrus, the bilateral superior frontal gyri, the thalami, and the right hippocampus (Grau-Rivera et al., 2020). It is an important finding considering the high prevalence of insomnia among older adults (Crowley, 2011) and the association between sleep fragmentation and elevated risk of AD (Lim et al., 2013).

Further, ε2 homozygotes and ε2/ε3 carriers reported lower odds of napping in comparison to ε3/ε3 carriers (Spira et al., 2017). Importantly, naps can be considered a manifestation of nocturnal sleep fragmentation and the duration of retrospectively reported naps in AD patients was shown to interact with APOE status (Asada et al., 2000). Naps longer than 60 minutes were related to a bigger risk of AD risk in ε4 carriers but not in non-carriers, whereas short naps (i.e., <30 minutes) were associated with decreased risk of incident AD in ε4 carriers and ε4 non-carriers (Asada et al., 2000).

Individuals with the ε4 allele reporting sleep disturbances (i.e., nocturnal awakenings, rising too early in the morning, napping extensively during the day) were at almost 7 times higher risk of developing AD compared to non-carriers (Burke et al., 2016). The risk of developing AD was shown to be 8 times higher in ε4/ε4 carriers with recent, self-reported depression, whereas clinically-verified depression increased the hazard by ten times (Burke et al., 2016). These findings suggest that sleep disturbances, episodes of depression, and APOE-ε4 status increase the risk of AD among initially cognitively asymptomatic individuals. It is an important notion considering the bi-directional relationship between depression and AD (Dafsari & Jessen, 2020), as well as between depression and

sleep (Feng et al., 2019). The association between APOE status and the risk of depression is less established due to inconsistent results (e.g., Burns et al., 2020, Ali et al., 2018).

Curiously, one study reported that non-demented older ε2 carriers demonstrated a greater frequency of poor nocturnal sleep quality (assessed by the Pittsburgh Sleep Questionnaire (PSQI)) compared to ε3 and ε4 carriers (Camargos et al., 2019). There was no significant association between the degree of reported extensive daily somnolence assessed by the Epworth Sleepiness Scale (ESS) and the APOE genotype. However, it is important to highlight that 81.6% of participants had diabetes mellitus and/or systemic arterial hypertension which could potentially confound associations of interest. Further, 47.8% of subjects took psychotropic agents, 26% were smokers and 9% was alcoholic which could further confound ESS and PSQI outcomes. However, the chi-square analyses revealed no frequency variation of these conditions across genetic groups. Further, the sample size of the ε2 carriers group was small (n=14) which calls for further investigation of reported associations.

Only one study reported a beneficial association between APOE-ε4 and self-reported sleep revealing less frequent snoring and sleep apnoea and no associations between ε4 allele carriership and the sleep quality or daytime sleepiness among non-demented older ε4 carriers (Tsapanou et al., 2017). Overall, the data on the association between the APOE-ε4 genotype and the risk of sleep breathing disorders is inconsistent (see review: Bliwise, 2002, meta-analysis: Lu et al., 2016). Notably, a recent study showed no significant associations between sleep apnoea and/or APOE polymorphism on levels of amyloid-beta in cerebrospinal fluid in cognitively healthy individuals (Hegde et al., 2020).

2.14.2. Actigraphy studies and APOE

It was reported that poor sleep characterized by actigraphy-assessed night-time wakefulness but no rest-activity changes were associated with an increased risk of dementia especially AD in 11.2 years long follow-up study (Lysen et al., 2020). Interestingly, stratified analysis by APOE-ε4 revealed that the relationship between increased risk of dementia and sleep outcomes holds only in ε4-negative individuals, however, no sleep-by-APOE ε4 interaction term survived multiple testing. Another actigraphy study showed that cognitively intact ε4 carriers had increased sleep fragmentation reflected by a higher number of awakenings compared to the non-carriers (Kahya, M., et al., 2018). Outcomes such as sleep latency, sleep efficiency, TST, and WASO were not significant. Subjective measures of sleep quality and daytime sleepiness have not differentiated across genotypes. Only the PSQI subcomponent of daily disturbances was significantly higher in the ε4 allele carriers. The authors suggest that disrupted sleep could be an early manifestation of AD that is present in already pre-symptomatic stages of the

disease. However, regardless of obtained large effect sizes, it is important to highlight a small sample size of the contrasted groups (n=9 APOE ε4 carriers and n=27 non-carriers).

APOE genotype was shown to modulate the relationship between actigraphy-measured TST and tau pathology (López-García et al., 2021). The interaction between TST and APOE-ε4 status significantly predicted tau levels and ε4 allele carriers were shown to have lower TST compared to non-carriers, though, no associations were found between memory performance and TST (López-García et al., 2021). Similarly, carriership of the ε4 allele was also shown to be associated with an increased risk of amyloid deposition in the preclinical stage of AD and was accompanied by decreased sleep efficiency (Ju et al., 2013). Further, Hwang et al., 2018 demonstrated that the ε4 allele had moderating effects on the associations between sleep latency (SL), Midline Statistic Of Rhythm (Midline Statistic Of Rhythm (MESOR))[25], and acrophase (timing of highest activity) with cerebral Aβ deposition in cognitively healthy adults. In ε4 non-carriers, shorter SL and higher mesor and advanced acrophase (i.e., circadian phase advance) were correlated with Aβ positivity. The authors suggested that advanced acrophase and shorted SL in ε4 non-carriers can be related to increasing sleepiness towards evening caused by the Aβ-related weakening of the circadian arousal signal in the wakefulness maintenance zone. Further, advanced acrophase in ε4 non-carriers and delayed acrophase in ε4 carriers were associated with elevated Aβ positivity. These changes in acrophase can be related to more progressed neurodegeneration in brain regions managing the sleep-wake rhythmicity which may be facilitated by earlier Aβ deposition in APOE-ε4 carriers or by another Aβ-independent neurodegeneration effect caused by the ε4 allele. A small number of ε4 allele carriers (n=25) and especially a very modest fraction of Aβ positive ones (n=8) compared to ε4 non-carriers (n=108, where 17 participants were Aβ positive) is worth considering while interpreting the results.

2.14.3. Polysomnographic studies and APOE

A significant relationship between the presence of the ε4 allele and objective sleep disturbances assessed by polysomnography and actigraphy was found in non-demented adults *without* subjective sleep complaints (Drogos et al., 2016). The risk allele (ε4+) carriers had shorter sleep duration, longer WASO and lower SE compared to non-carriers. Further, after correcting for age and sex, ε4 carriers had a significantly lower percentage of total sleep time in N2 (5.4% lower), and significantly more time spent in REM (4.1% more) sleep compared to ε4 non-carriers. Note, that the 4.1% increase in the amount of time spent in REM sleep is not clinically significant because outcomes of both groups were still close to normative data for individuals in the age group between 60 to 69 years. Notably, 51.9% of non-carriers

[25] MESOR is the rhythm-adjusted mean that is the average value of the cosine curve fitted to the data. See Methods, for more information.

and 37.5% of ε4 carriers reached an apnoea-hypoxia index exceeding 15 qualifying them for clinical diagnosis of sleep apnoea. This in turn poses a question of how much of the reported sleep alterations can be attributed to ε4 carriership status and how much to sleep apnoea.

The interaction between the ε4 allele and the percentage of REM sleep was shown to be a predictor of AD incidence. Stratification of the results revealed that lower REM sleep percentage was associated with a higher risk of AD incidence in ε4 non-carriers but *not* in carriers (Pase et al., 2017). Yet, the duration of REM was found to be significantly reduced in MCI patients with ε4+ status (Hita-Yañez et al., 2012). Further, Tranah et al., 2018 showed that ε4+ carriership was associated with increased duration of SWS in elderly men. Total time in SWS sleep was significantly higher in ε4 homozygotes (62±5.2 minutes) compared to heterozygotes (43±1.5 min) and ε4 non-carriers (40±0.8 min). Carriership of the ε4 allele was also significantly associated with lower cognitive function scores (Modified MMS assessment). Also, Drogos et al. (2016) reported a trend toward significance for an increase in the amount of time spent in SWS in ε4 carriers compared to non-carriers. Increased time in SWS might suggest overactive synaptic downscaling and increased sleep pressure (Tononi&Cirelli, 2006). This is supported by evidence showing a negative association between the increased duration of SWS and episodic memory consolidation in the elderly (Sculin, 2013) and in people with accelerated long-term forgetting due to epilepsy (Atherton et al., 2016). Further, higher cortical Aβ pathology in healthy older adults was shown to be associated with the disrupted generation of SWS (Mander et al., 2015). Interestingly, Muto et al. (2020) showed no difference in slow-wave energy (SWE) and daytime sleepiness between ε4 carriers and non-carriers in a cohort of men in their early twenties which suggests that age can play a role in modifying the properties of SWS.

Crucially, greater sleep consolidation was shown to reduce the effect of the ε4 allele on the increased annual rate of cognitive decline and neurofibrillary tangle pathology (Lim et al., 2014). Since the paper was published, several groups have tried to develop interventions aiming to improve sleep consolidation (review paper: Grimald et al., 2020) but there is no consensus if state-of-art neurostimulation techniques such as auditory closed-loop stimulation should be used in the clinical AD population.

SUMMARY – Sleep and Circadian Rhythmicity

Sleep is regulated by circadian rhythm (Process C) and sleep-wake homeostasis (Process S) (Borbély et al., 1982). The two components of the sleep-wake regulatory system are neuroanatomically distinct but work in dynamic interaction in defining the timing, duration and quality of sleep and wakefulness. Further, sleep consists of NREM (N1, N2, N3) and REM stages and is heterogenous from a physiological standpoint as each sleep stage is characterised by different physiological patterns of the electrical brain (EEG), eyes (EOG) and muscle activity (EMG) (Berry et al., 2020).

Sleep physiology changes across the lifespan and its alterations, for example, shorter duration of deep sleep (N3) is commonly seen in older age (e.g., Mander et al., 2013). Further, sleep alterations were reported to worsen with progressing AD neurodegeneration which highlights a bidirectional relationship between progressing brain atrophy and disturbed sleep (e.g., Ju et al., 2013, Bubu et al., 2017). Likewise, 24-h activity rhythms are affected by ageing, for instance, older adults present usually an advanced phase sleep pattern, while circadian abnormalities are associated with an increased risk for dementia (e.g., Musiek et al., 2018, Tranah et al., 2011). Accordingly, sleep and circadian rest-activity alterations might serve as potential early markers of neurodegenerative diseases.

Notably, the associations between a major genetic risk factor for developing late-onset Alzheimer's disease, i.e., APOE-ε4 allele carriership, sleep-wake homeostasis and the circadian system remains unclear as the published studies demonstrated heterogeneous results (e.g., Asada et al., 2000, Drogos et al., 2016, Tranah et al., 2018, Kahya, M., et al., 2018, Camargos et al., 2019). Furthermore, it was proposed that spatial navigation deficits are an early cognitive marker of AD (e.g., Coughlan et al., 2018). It is, however not known how the APOE-ε4 allele, time of the day and experimentally manipulated sleep pressure impact spatial navigation performance which highlights a research gap addressed in the presented PhD thesis. Therefore, the following chapter will focus on the impact of APOE polymorphism on spatial navigation and its associations with sleep.

CHAPTER III. Spatial navigation

3. 1. Neuronal bases of spatial navigation

Spatial navigation is a process of assessing accurately one's position in space and sustaining a planned trajectory between different points in the environment (Coughlan et al., 2018). How does the brain create the internal representation of a space that allows an organism to navigate? The neural implementation of spatial navigation consists of the following spatial components of the hippocampal formation: place cells (O'Keefe, 1976), grid cells (Hafting et al., 2005), boundary cells (Solstad et al., 2008) and head direction cells (Taube et al., 1990).

Place cells and grid cells are involved in the generation of spatial maps that mirror representations of an environment (O'Keefe & Dostrovsky, 1971). The firing pattern of the place cells creates an internal neural map of the explored environment, whereas the hexagonal pattern of grid cells provides a metric to the map, i.e., distance perspective. The other correlates involved in the complex mechanism of spatial navigation are head direction cells activated when the head points in a certain direction (like a compass) and border cells firing in presence of walls and other kinds of boundaries (Solstad et al., 2008, Taube et al., 1990). In addition, the brain needs to estimate the distance between the objects which can be accomplished by object-sensitive cells. The visual processing of objects is executed via spatial, dorsal streams (where?) and nonspatial, ventral, streams (what?) (Ward, 2020). The act of navigation is driven by an aim to reach a planned goal destination. Accordingly, another type of cell is the goal cell that encodes spatial and non-spatial representations of the target destination, for example, a representation of a bakery allows efficient and flexible navigation instead of a repetitive exploration of the environment each time when one needs to buy a baguette (Grieves & Jeffery, 2017).

Sophistically shaped spatial maps are subsequently consolidated for further usage, i.e., retrieval. A groundbreaking case of patient H.M. (Squire, 2009) showed that a lesion of the hippocampus that caused anterograde amnesia[26] lead also to impairment in spatial navigation to the extent that H.M. was not able to read maps to navigate in an unfamiliar environment (Corkin, 2002). The case of H.M. caused a burst of interest regarding the hippocampus as the key area of memory and spatial navigation. The rapid advancement of neuroimaging techniques demonstrated that both processes are much more complex and involve more brain areas than previously believed (Table 3). Crucially, the complex patterns of firing that define the position of an individual and surrounding objects can be stored in memory allowing an individual to come back home or to find the best pizzeria in the city in a fast, efficient and flexible way. Accordingly, the brain can store patterns that indicate important locations allowing an

[26] *anterograde amnesia* - an inability to create new memories after the event that lead to amnesia

individual, for instance, to recall a certain location in comparison to the pattern of a current location (or any other position) which allows, in turn, to work out the distance between locations and obey the direction that needs to be followed to reach a goal destination (Grieves & Jeffery, 2017).

	Type of cells underlying spatial navigation	The function	Location in a brain
Functional cell types underlying processes of cognitive mapping	Place cells	different place cells fire at different spatial locations in an environment (place field) which allows the formation of cognitive maps	hippocampus, nucleus reuniens, parataenial nucleus, anteromedial nucleus, claustrum, medial entorhinal cortex and subiculum
	Grid cells	provides metric information (i.e., distance estimation)	medial entorhinal cortex, pre- and parasubiculum
	Head direction cells	cells responding exclusively to the direction where the animal is facing; plays the role of the compass of a brain contributing to encoding orientation in a space	mamillary nuclei, anterodorsal nuclei, laterodorsal nuclei, retrosplenial cortex, postsubiculum, nucleusreuniens and anteromedial nucleus
Chosen types of other spatially modulated neurons	Place correlates	cells exhibiting weak spatial activity	orbitofrontal cortex, postrhinal cortex, lateral entorhinal cortex and lateral septum
	Boundary/border cells	respond exclusively to environmental boundaries	parasubiculum, claustrum, subiculum, anterior cingulate cortex, pre-and parasubiculum and medial entorhinal cortex
	Object sensitive cells	responsible for discrimination of non-spatial cues such as objects recognition	lateral entorhinal cortex, postrhinal cortex, orbitofrontal cortex and the lateral septum
	Goal cells	encode a representation of spatial goals facilitating efficient and flexible navigation	medial prefrontal cortex and prelimbic and infralimbic regions of the prefrontal cortex
	Self-motion and Egocentric cells	code the running speed or angular head velocity	the medial entorhinal cortex, striatum, Retrosplenial cortex, Posterior Parietal Cortex, Lower Motor Neuron and Dorsal tegmental nucleus

Table 3. The neural basis of the representation of space. The content of the table is based on Grieves & Jeffery, 2017.

3.2. Navigation strategies

The behavioural outcome of the complex interaction between the above-mentioned spatial sensitive cells is the application of *spatial strategies*. Efficient spatial navigation is crucial for survival in the Animal Kingdom. Animals can explore widespread territories in search of food, water or mates and be able to navigate back to the shelter or another desired location encoded in the cognitive map (Grieves & Jeffery, 2017). Humans are extraordinary navigators whose curiosity and evolutionary pressures lead them to travel across lands and seas for centuries. How the brain can explore an environment systematically and efficiently?

Spatial navigation relies on two liaising coding strategies, i.e., the *allocentric*, world-centred hippocampal-dependent strategy and the *egocentric*, self-centred strategy (Figure 9). The egocentric navigational strategy requires visual input to estimate the body position relative to the local landmarks, further, sensorimotor and vestibular stimuli provide information about the location in space, while, the temporal order of the spatial cues facilitates the landmark-based behaviour (Coughlan et al., 2018). The egocentric strategy is difficult to apply in unfamiliar environments because of its dependence on local and well-known landmarks to navigate; however, it makes it very effective in familiar locations (Yesiltepe et al., 2021).

Allocentric spatial navigation relies prominently on the place cells that are involved in the encoding of a new environment. The place cells become more stable and more spatially restricted once an individual becomes familiar with an environment by creating *cognitive maps* (Coughlan et al., 2018). Cognitive maps allow obtaining, storing and recalling information about the position in space which allows a navigator to create an internal representation of the environment. An individual can identify current self-location and update the allocentric representation without reference to external cues via *path integration* that combines information from visual, vestibular and proprioceptive systems[27] (Coughlan et al., 2018). Grid cells are key neural correlates of the network underlying path integration because of their properties to encode self-location and measure the distance between positions. Path integration is a mechanism via which a brain can recognize a current position in a space based on sensory information of self-motion (provided by the cells encoding specifically for self-motion) to update the length of travelled distance (conveyed by boundary vector cells) and be able to come back to the starting location.

[27] Vestibular system and sense of proprioception convey information about the movement and orientation of one's body in the space.

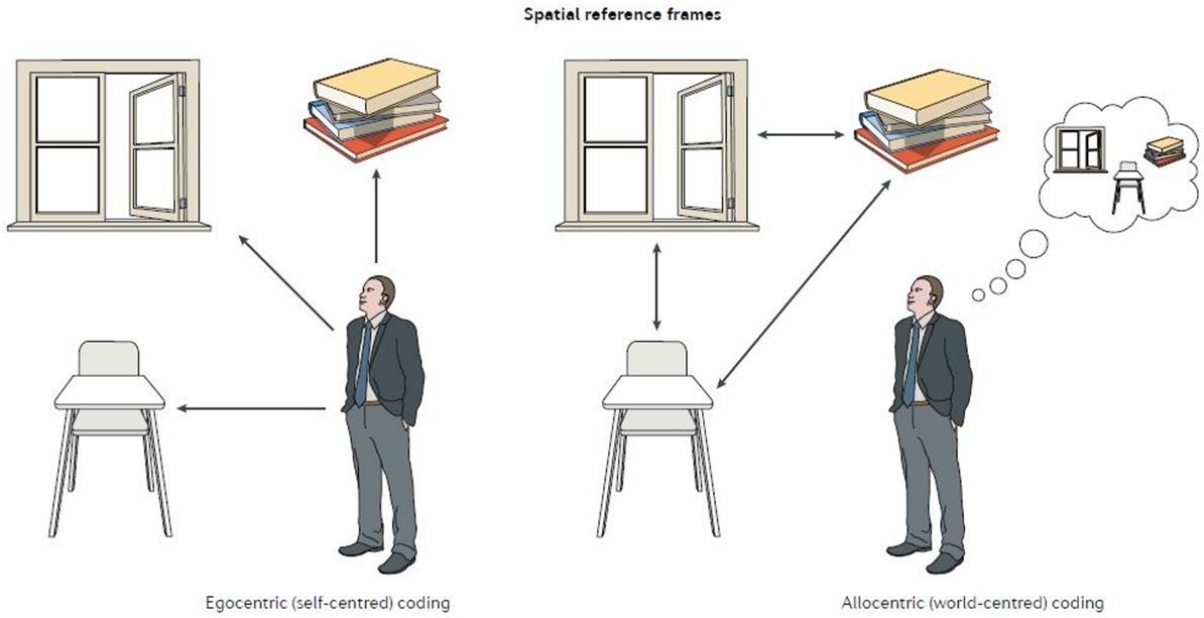


Figure 9. Spatial navigational strategies. Egocentric strategy encodes spatial clues from the perspective of the navigator and is usually applied when one is following familiar routes that do not require the involvement of conscious control. Allocentric navigation is usually used in an unfamiliar and/or large environment, where a person relies on the perception of landmark positions relative to other landmarks. Figure copied from Coughlan et al., 2018.

The posterior parietal cortex mediates egocentric viewpoint-dependent navigation, whereas the medial temporal lobe mediates allocentric, viewpoint-independent frames (Vann et al., 2009). The retrosplenial cortex was proposed to transform allocentric representations into egocentric representations and vice versa (Byrne et al., 2007). The retrosplenial cortex uses head direction information to compensate for the so-called *rotational offset* between egocentric and allocentric coordinates (Byrne et al., 2007). Practically, when a brain creates an allocentric framework between a goal location and a landmark located 10m towards North (orientation in space is encoded via "*brain-compass*", i.e., head-direction cells), this representation needs to be converted into the egocentric framework to enable an individual to take a motor action by taking right or left turn respectively to the current standpoint.

3.3. Spatial navigation and ageing

Spatial navigation is one of the first cognitive functions to decline sharply with age which can be associated with age-related hippocampal volume reduction (Schuff et al., 2009). Recently, *noisy hippocampal activity* was suggested to be a culprit of navigational impairment attributed to ageing (Diersch et al., 2021). An fMRI study, where the participants needed to learn a layout of a photorealistic Virtual Reality (VR) town revealed age-related cognitive mapping deficits in older adults compared to

younger ones. The discrepancies were associated with increased anterior hippocampus *excitability*. Healthy older adults demonstrated significant problems with learning how to navigate in a new VR-based city and experienced difficulties when locations were encountered from new directions. These difficulties suggest the deficits in discrimination between novel and familiar information and an alteration in allocentric processing.

Further, reduced grid-cell-like representations in the entorhinal cortex which can be caused by temporal instability of spatial representation or insufficient spatial stability are associated with path-integration deficits in elderly adults (Stangl et al., 2018). Importantly, without a harmonious, temporally precise firing pattern of the grid cells, the interplay between EC and hippocampus will be impaired which can lead to deficits in the computation of self-position, which in practical terms will lead to worse performance in path integration tasks such as the Object-Location Memory Task.

Elderly rats show more prominent alterations in allocentric than in egocentric tasks, moreover, after being successfully trained on both allocentric and egocentric strategies they use preferentially egocentric responses (Barnes et al., 1980). Likewise, older humans perform worse in allocentric compared to egocentric tasks (Fernandez-Baizan et al., 2019), and, favour the egocentric strategy to complete navigational tasks (Colombo et al., 2017). Older adults were shown to shift between allocentric to egocentric strategy more efficiently than switching from egocentric to the allocentric frame of reference (Harris & Wolbers, 2014) which indicates a compensatory shift favouring egocentric navigation (Coughlan et al., 2018). These age-related strategy-switching alterations were suggested to be caused by changes in locus coeruleus (LC) functioning (Lester et al., 2017). Notably, ageing is associated with a significant loss of neurons in the LC and its integrity varies considerably across healthy older individuals (Hämmerer et al., 2018)[28].

Importantly, age-dependent changes in navigation strategies were shown to be manifested in midlife, for instance, midlife adults used significantly fewer shortcuts in the dual-solution paradigm compared to young adults (Yu et al.,2021). This is consistent with previous studies showing that with advancing age, individuals favour using habitual routes while navigating (Lester et al., 2017) which is further in line with age-related gradually increasing levels of self-reported spatial anxiety (especially in unfamiliar places) (van der Ham, 2020). Further, in the path integration task, middle-aged adults were less successful with route learning than young individuals, despite equal exposure to the route (Yu et al.,2021). It was also shown that young and old adults indicated comparable self-reported navigation

[28] Moreover, AD-tau neurofibrillary tangles deposition begins in the LC and cholinergic brainstem nuclei in Braak stage 0, progressing into entorhinal cortex in the medial temporal lobe by stage I (Braak & Braak, 1991) what supports spatial navigation alteration as early cognitive marker of AD.

ability which suggests that older individuals overestimated their skills as spatial navigation is known to decline with age (Tailade et al., 2016, van der Ham, 2020).

Understanding the trajectory of spatial navigation decline in healthy ageing and the development of diagnostic methods that discriminate between ageing-attributed decline and decline due to AD is crucial for establishing AD-specific cognitive markers. However, the brain's navigational circuit involves a bunch of complex cognitive operations, therefore, age-related deficits can originate at numerous *navigational processing stages* (Lester et al., 2017). The navigational alteration can arise from computing spatial information obtained via sensory signals (e.g., self-motion processing), through problems with coding stable spatial memory traces (learning) to difficulties with planning and monitoring navigation (Lester et al., 2017). Despite a large number of studies on the topic of spatial navigation and ageing in humans, there is a very small number of longitudinal studies that prevent uncovering solely age-related changes which in turn makes it even harder to indicate precise neurodegenerative navigational alterations.

3.4. Spatial navigation – clinical significance

Spatial disorientation is the impairment when an individual becomes lost even in a familiar environment and is a characteristic feature of early AD. In both animal and human-based models, the brain's navigation system corresponds significantly with the regions affected by AD (Coughlan et al., 2018). The brain areas altered initially by neurodegenerative processes associated with AD pathophysiology are the main neuronal correlates of the spatial navigation network (Figure 10) (Fu et al., 2017, Serino et al, 2017, Pengas et al., 2012, Jheng & Pai, 2009, Serino, S. & Riva, 2013, Irish et al., 2015).

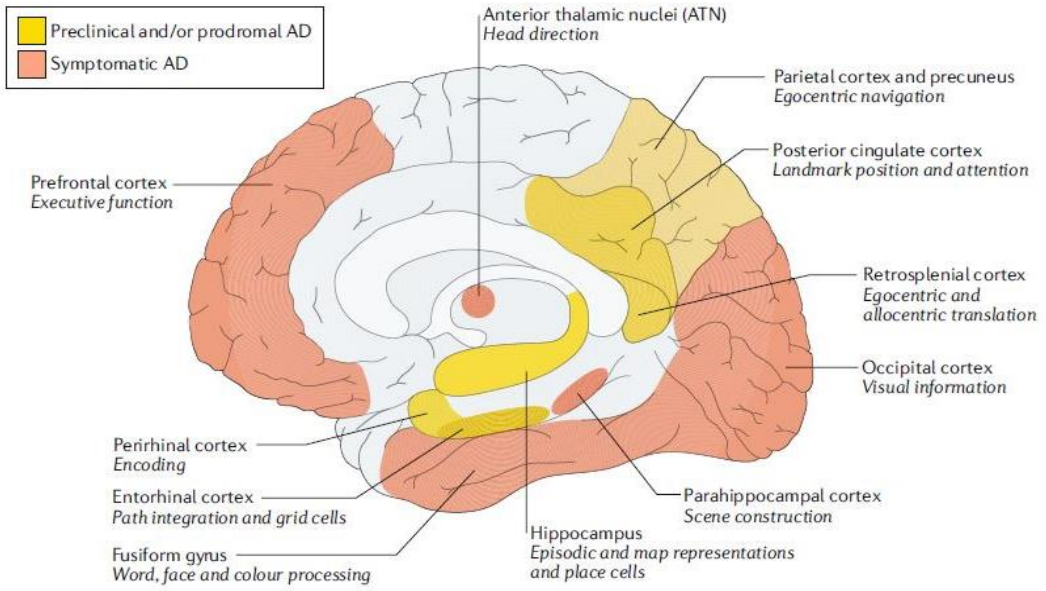


Figure 10. Spatial navigation – clinical significance. The illustration presents the overlapping brain areas involved in spatial navigation and neuropathological changes caused by Alzheimer's Disease in pre-symptomatic (areas highlighted in yellow) and symptomatic stages. Figure copied from Coughlan et al., 2018.

Accordingly, it was proposed that spatial navigation deficits might serve as an early cognitive marker of AD (Gazova et al., 2012, Coughlan et al., 2018, Howett et al., 2019, Levine et al., 2020). Notably, the episodic memory tasks commonly used in clinical practice demonstrate good sensitivity yet poor specificity to early detection of AD (Coughlan et al., 2018). Episodic memory problems are prevalent in healthy ageing, while episodic memory deficits are a common symptom of several neurodegenerative diseases (e.g., a behavioural variant of frontotemporal dementia) (Yew et al., 2013; Hornberger et al., 2010). Spatial disorientation in a familiar environment, on the other hand, is characteristic of AD and is common neither in healthy ageing (Lithfous et al., 2013) nor in other dementias. MCI patients with positive CFS AD markers (individuals at high risk of developing AD) were shown to perform worse on the entorhinal cortex-based VR path integration task[29] compared to negative CSF markers individuals (individuals at lower risk of developing AD) (Howett, et al., 2019). Importantly, the designed task obtained better results in discriminating between participants at higher and lower risk of developing AD than gold standard neuropsychological tests which are used nowadays for early diagnosis of AD.

Furthermore, tasks assessing spatial navigation are affected to a lesser extent by verbal proficiency and cultural biases (Coughlan et al., 2018) which allows comparison of individuals across the

[29] Path integration was tested using an immersive virtual reality task where participants were asked to navigate real-world environments in VR where environmental cue, e.g., boundary cues were manipulated.

world. Moreover, in comparison to episodic memory, spatial navigation can be easily assessed in animals which enables easier translation of the obtained outcomes to humans.

3.5. Spatial navigation and APOE genotype

3.5.1. Effect of APOE polymorphism on spatial navigation in a clinical population

APOE status was shown to impact spatial navigation in patients with Amnestic Mild Cognitive Impairment (aMCI)[30] regardless of demographics and cognitive profile (Laczó et al., 2014). aMCI APOE-ε4 allele carriers performed significantly worse on a computerized version of the Human Analogue of the Morris Water Maze test, in a dose-dependent manner, compared to aMCI ε4 non-carriers. The difference in performance was independent of age, biological sex, years of education, and degree of verbal and nonverbal memory impairment (Laczó et al., 2014). Egocentric navigation was particularly impaired in ε4 homozygotes, whereas outcomes of allocentric navigation were not significantly different between ε4 carriers and non-carriers. On a structural level, APOE-ε4 homozygous showed a reduction of right hippocampal volume. Further, at-risk individuals (ε4 allele carriers (ε4+)) who reported Subjective Spatial Navigational Complaints and Subjective Memory Complaints[31] were shown to have brain atrophy in the regions involved in spatial navigation, i.e., cortical thinning in the precuneus and parahippocampus (Nedelska et al., 2015). Importantly, aMCI and Subjective Memory Complaints are the known incipients of AD which strongly suggest that an assessment of spatial navigation can serve as a promising screening tool for the early stages of AD.

3.5.2. Effect of APOE genotype on spatial navigation in healthy individuals

APOE polymorphism was reported to affect spatial navigation performance and the choice of navigational strategies to complete the task in the cognitively intact participants. Healthy, at-risk individuals (ε4+) demonstrated altered spatial navigation compared to APOE-ε3 homozygous (Coughlan et al., 2018). Cognitively intact non-ε4 carriers outperform ε4 carriers playing the Sea Hero Quest (SHQ) task (Coutrot et al., 2018)[32]. At-risk individuals took a longer wayfinding distance in the allocentric task and were biased in navigating toward the borders of the SHQ environment. The longitudinal assessment (18 months after the baseline visit) demonstrated that boundary-based navigation predicts the degree of subjective episodic memory complaints in ε4 carriers exclusively (Coughlan et al., 2020). The authors

[30] Amnestic Mild Cognitive Impairment – represents a noticeable and measurable cognitive impairment that primarily affects memory. Diagnosis of MCI increases risk of developing AD or other form of dementia.
[31] Subjective Memory Complaints (SMC) - a subjective perception of *subtle* changes in memory when objective impairment in memory is not confirmed.
[32] In the SHQ task, in the allocentric part, the participants were asked to encode a map with highlighted checkpoints and then navigate a boat to reach them in an ascending order. The egocentric task required exploring the environment to find a flare gun and shut the flare towards the starting point. See the Methods section of this thesis for more information.

suggest that this *border bias* is related to navigational uncertainty in ε4 carriers which can be caused by errors in the EC grid cells system which measures travelled distance allowing the continuous encoding of the representation of self-location (Coughlan et al., 2018). Moreover, navigating along the borders does not promote taking shortcuts which further contributes to worse wayfinding performance because of taking a longer route. In line with Coughlan et al., 2018, it was reported that young adults with APOE-ε3/ε4 genotype had reduced grid-cells representation relative to APOE-ε3/ε3 which was demonstrated by impaired connectivity between the right EC and hippocampus (Kunz et al., 2015). In the Kunz et al., 2015 study, the participants completed an object-location memory task that required navigating in the circular VR environment. No differences in spatial memory between the at-risk and control group were found. There was, however, a significant difference related to behavioural changes. At-risk individuals showed a preference for navigating along the borders (i.e., reduced central navigational preference). That can be explained again by the reduction of the grid cells-like representation that leads to impaired distance estimation.

3.5.3. APOE-associated changes in a spatial navigational network

The study by Kunz et al., 2015 highlights that neurophysiological changes in grid cells can be present already in early adulthood. Notably, in the transgenic mice model of AD, it was demonstrated that tau accumulation impairs grid cell activity leading to spatial memory deficits (Fu et al., 2017). Elderly mice with EC-tau pathology showed grid cell hypoactivity and reduced periodicity (Figure 11, Fu et al., 2017). The authors proposed that the formation of tangles in the EC occurring at the earliest stages of AD could potentially lead to alterations in grid cell firing which can initiate navigational impairment in humans with AD.

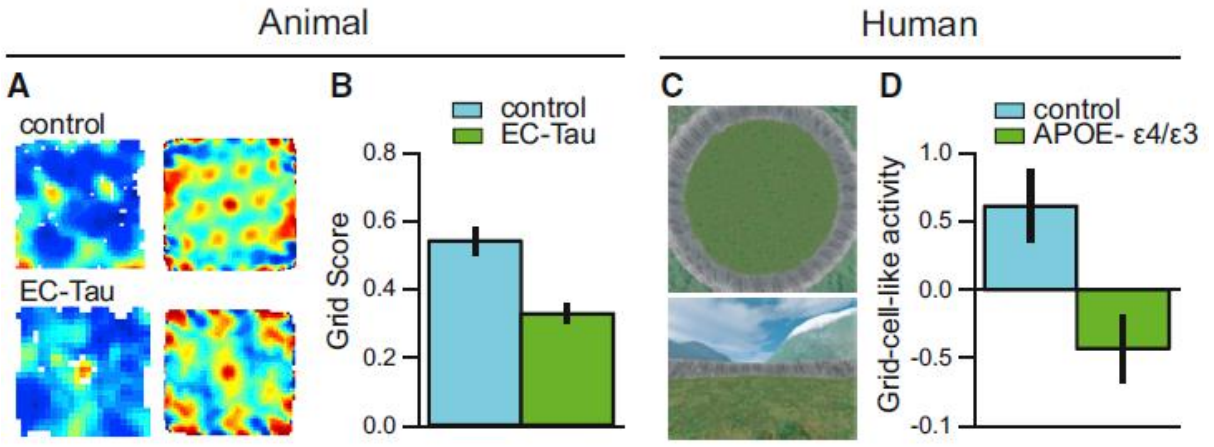


Figure 11. Grid Cell Alterations in transgenic mice model of Alzheimer's Disease (Fu et al., 2017) and cognitively intact young humans (Kunz et al., 2015). A. Impair grid cells functioning in entorhinal cortex-Tau positive elderly mice can be seen on the firing rate maps and B. grid score (i.e., spatial periodicity). C. The screenshots show the virtual reality environment used to test gird cell functioning in young humans, where the controls are APOE-ε3/ε3 carriers. The fMRI-obtained grid cell activity in the entorhinal cortex demonstrates altered grid cell-like representations among ε4 carriers. Source of the figure: Lester et al., 2017

Furthermore, APOE-ε2 allele carriers were shown to use a significantly more often hippocampus-dependent strategy to navigate in a virtual environment compared to ε3/ε3 and ε4 individuals, who used mostly a caudate nucleus-dependent response strategy (Konishi et al., 2016). Strikingly, ε2 carriers had significantly more grey matter in the hippocampus compared to ε3 homozygous individuals and ε4 carriers. It was hypothesised that a higher volume of grey matter in the hippocampus found in ε2 individuals can play a protective role by resisting more effectively age-related cortical atrophy before any cognitive symptoms occur (Konishi et al., 2016). The strong preference for using the hippocampal-dependent spatial strategy by the ε2 carriers was suggested to stimulate the hippocampus (*hippocampal training*). This concept is in line with the studies showing that spatial memory training led to an increased volume of hippocampal grey matter in mice (Lerch et al., 2011), as well as elderly individuals who trained to increase the use of spatial strategy (Bohbot et al., 2015).

Results by Konishi et al. (2016) contribute to a modest body of evidence highlighting the association between structural brain differences in the hippocampus and APOE polymorphism. Notably, the left EC was shown to have a greater cortical thickness in children and adolescents who were ε2 carriers compared to ε3/ε3 and ε4[33] (Shaw et al., 2007). Adults demonstrated corresponding results (Fennema-Notestine, et al., 2011). Importantly, brain atrophy in AD starts in the EC (Braak & Braak, 1995). The thinner EC can potentially contribute to an elevated risk of AD in ε4+ individuals, who show also decreased hippocampal volume compared to ε4- (e.g., Heijer et al., 2002, Crivello et al., 2010). These volume differences in the hippocampus and its subfields can serve as a potential early marker of AD, especially when we consider the particular vulnerability of the hippocampus towards age-related atrophy and its volume differences associated with female sex[34] and APOE status (which combined are the strongest predictors of AD).

A recent study on healthy elderly people reported a significant association between APOE genotype, sex and age on the volume of the whole hippocampus and its subfields (Veldsman et al., 2021). Strikingly, the data derived from the UK Biobank showed that the entire hippocampal volume is gradually reduced starting from 50 to 65 years of age, and then, it is followed by a much more abrupt reduction, especially in women which can be related to post-menopause ovarian hormonal changes[35] (Veldsman et al., 2021). The most severe hippocampal volume loss, especially in the presubiculum, subiculum head, cornu ammonis 1 body, cornu ammonis 3 head and cornu ammonis 4, was seen in ε4

[33] There was a significant stepwise growth in cortical thickness in the left EC regions, with ε4 carriers having the thinnest cortex and ε2 carriers the thickest, with ε3 homozygotes located in between.
[34] Importantly, among brain regions that are particularly sensitive towards alterations of sex steroids hormones (e.g., menopause-promoted hormonal changes) are the hippocampus, entorhinal cortex and prefrontal cortex which are the key regions in spatial navigation networks (Hara et al., 2012, Taylor et al., 2020, Jacobs & Goldstein, 2018).
[35] It is suggested that ovarian hormones are considered to have neuroprotective effects (Vegeto et al., 2020).

homozygote females aged ≤ 65. Thus, the study provides an estimate of a critical time window indicating the approximate time point when hippocampal volume loss is likely to accelerate. Notably, the other medial temporal lobe regions and subcortical regions have not demonstrated similar vulnerability. The outcomes show that older females who are ε4 homozygotes are particularly vulnerable to hippocampal volume loss which highlights how important is to acknowledge menopausal changes while conducting studies involving the elderly population. There is a research gap in understanding the impact of menopause-induced hormonal changes on spatial navigation. It is a vital area as female sex and age are the non-modifiable risk factors of AD, hence, the menopausal transition can potentially be a sex-specific risk factor for AD (Mielke et al., 2014) while spatial navigation deficits were proposed to be an early marker of preclinical AD (Coughlan et al., 2018).

3.6. Spatial navigation and sleep

Most of the studies cited in this sub-chapter focus on the impact of sleep on *spatial memory* and *spatial learning.* Accordingly, a clear distinction between spatial memory and spatial navigation must be emphasised. Spatial navigation is a complex cognitive skill that involves the processing of visual input, the temporal order of environmental stimuli, the distance of a body from landmarks, sensorimotor and vestibular information regarding the position in space, and uses spatial memory *to determine and keep a trajectory between different locations in the environment* (Coughlan et al., 2018). Spatial memory (i.e., spatial working memory, spatial short-term memory, spatial long-term memory), on the other hand, is a form of memory that encodes and retrieves information needed to plan a path to a given location, therefore, spatial memory is essential for navigation in space. Spatial learning involves the rapid encoding of novel environmental features which are then replayed by hippocampal cells during subsequent sleep, i.e., the replayed pattern of activity mirrors the one activated by spatial learning (Skaggs & McNaughton, 1996, Peigneux et al., 2004). In a presented PhD project, the main focus was what is the effect of sleep pressure and time of the day on *spatial navigation,* not on spatial memory which highlights the novelty of the presented PhD thesis.

3.6.1. Consolidation of spatial memories during sleep

Sleep is crucial for incorporating recently learnt memories into long-term memory storage. Memory reactivation during sleep consolidates initial hippocampal memory representation to the long-term neocortical representation. This information transfer is facilitated by hippocampal sharp-wave ripple complexes that are timely coordinated with neocortical slow weaves (SWS) (Sirorta et al., 2003). Importantly, learning itself induces several micro-and macrostructural changes in post-learning sleep to facilitate offline memory consolidation (Rash & Born, 2013). The EC-hippocampus system plays a key

role in spatial memory consolidation and their interplay is especially active during SWS (Hahn et al., 2012, Figure 12).

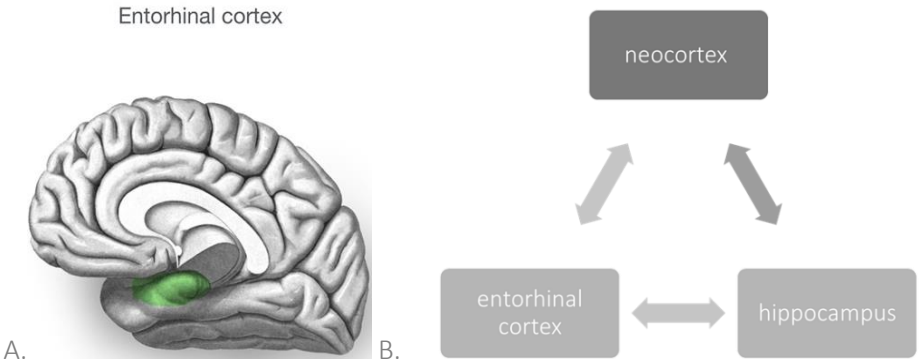


Figure 12. Consolidation of spatial memories during sleep – the significance of the entorhinal cortex (EC). The EC is an important player in the communication between the neocortex and hippocampus that takes place during slow-wave sleep. A. EC (green highlight) is a part of the medial temporal lobe (MTL) and plays a role of the main input and output of the hippocampus. It is also a main crossing point between the hippocampus and neocortex. B. The graph presents the involvement of EC neurons in a neocortex-hippocampal dialogue engaged in learning and memory consolidation during sleep. Source of image brainfacts.org

A novel study showed that genetically knockout mice of adult hippocampal neurogenesis[36] which performed three-day-long training in the Morris water maze (MWM) task had altered sleep micro-and macrostructure and impaired sleep-specific neuronal oscillations involved in memory consolidation (Sippel et al., 2020). The authors proposed that adult neurogenesis is a key event of hippocampal plasticity that not only can be involved in sleep-dependent memory formation but also modulate learning-induced changes in sleep. While performing the MWM task the mice lacking adult hippocampal neurogenesis have shown no impairment of the maze encoding, they barely used the most effective hippocampus-dependent search strategy. The mice also demonstrated poor route efficiency while performing a task right after the first sleep following learning. Sleep-wise, during the baseline night, the genetically knockout mice had reduced total sleep time, higher arousal index, shorter NREM sleep duration and generated significantly more slow oscillations (count) and sleep spindles (count) compared to the control mice. Once the mice were trained on the MWM task, all of them had increased duration of NREM sleep and spindle count suggesting spatial memory consolidation. Notably, the performance on the MWM and sleep parameters following learning showed that longer NREM sleep duration and higher sleep spindle density (a hallmark of sleep-dependent memory consolidation) were correlated with better performance but *only* in the control mice.

[36] Neurogenesis refers to the formation of new neurons which fit into existing circuits. In an adult animal brain, neuroanatomical landmarks of neurogenesis are the lateral subventricular zone and the dental gyrus of the hippocampus (Kumar et al., 2019).

Another rodent study showed that overactivity of the LC[37], during sleep, interferes with sleep features associated with sleep-dependent memory consolidation, i.e., reduction in sleep spindles occurrence, power in theta band during REM sleep and ripple-spindle coupling (Swift et al., 2018). Abnormal activity of LC during sleep obtained by optogenetic stimulation of norepinephrine LC neurons following hippocampus-depended food location task lead to impaired consolidation of encoded localization the next day. The authors pointed out that external LC stimulation has not affected hippocampal ripples or the activity of pyramid cells. Yet, the stimulation reduced the ability of hippocampal place encoding, consequently, the next day, after the night of LC stimulation, the rats were using non-hippocampal, procedural strategies while performing spatial navigation tasks. The study suggests that periods of LC silence during sleep following learning are critical for synaptic plasticity related to normal spindle generation, delta and theta power, and efficient hippocampal consolidation of spatial memories. A potential study on humans could further investigate if there is an association between LC functional alterations and/or LC integrity (especially due to age-associated LC alteration) and cognitive decline, especially in spatial navigation and how it interacts with sleep architecture.

3.6.2. Effect of sleep on spatial navigational performance

One of the first ground-breaking studies aiming to investigate the *mechanistic* association between sleep and spatial learning revealed that the pattern of rat's brain cells firing recorded during learning of the spatial maze layout was replayed during subsequent sleep (Skaggs & McNaughton, 1996). The very same phenomenon was shown in humans, namely, the hippocampal areas that were activated during the wayfinding encoding task were triggered likewise following slow-wave sleep (SWS) (Peigneux et al., 2004). The amount of hippocampal activity during SWS was positively correlated with improvement of the route recall on a subsequent day.

Interestingly, the non-REM pattern of hippocampal activity replay was significantly faster than hippocampal activity during wake (encoding) (Skaggs & McNaughton, 1996), while the REM replay temporal resolution was more similar to wake (Louie & Wilson, 2001).

Overnight sleep was shown to improve accuracy to navigate to the goal destination in a virtual maze task (Nguyen et al., 2013). Individuals were trained on the virtual maze navigational task at either 10:00 AM (wake group) or 10:00 PM (sleep group) and were tested 11h later. On the following morning, only the participants who received an opportunity of a full night of sleep enhanced the speed of the maze competition by following a more accurate route to reach the goal destination (measured by the distance travelled and backtracking). The overnight improvement of recently learnt spatial mapping of the maze was attributed to the consolidation of hippocampus-dependent spatial information.

[37] During normal, healthy sleep, the activity of LC is reduced during spindles and completely silent during REM sleep.

Surprisingly, PSG results revealed that neither the total amount of sleep nor time spent in any given sleep stage could predict performance on the morning retest.

Further, young adults reporting poor *subjective sleep* quality were shown to be slower and more error-prone in a wayfinding task in a virtual environment in comparison to those reporting good quality of sleep (Valera et al., 2016). There were no differences in objectively, actigraphy-assessed sleep quality. Another study using actigraphy revealed that sleep fragmentation in cognitively healthy elderly adults (age <60) was associated with poorer hippocampal-dependent performance in a dual-solution virtual maze task but not the striatal-dependent navigation[38] (Maybrier et al., 2020). Shorter total sleep time was associated with poorer cognitive mapping, but there was no association with route learning. Interestingly, longer sleep time was associated with favouring striatal strategy which is surprising considering that sleep preferentially enhances the formation of hippocampus-dependent memory (e.g., Noack et al., 2017). It was also shown that the degree of REM fragmentation caused by obstructive sleep apnoea was associated with an altered overnight enhancement in spatial navigation tasks (Varga et al., 2014).

Further, a study by Varga et al. (2016) showed that overnight spatial navigation performance was improved significantly more in young compared to elderly participants (31% to 4.8% gain in a virtual maze). The frontal slow-wave activity was positively correlated with overnight gain in maze performance as well as medial prefrontal cortical volume. Importantly, older participants demonstrated significantly shorter slow-wave sleep and longer overnight maze competition time compared to young subjects.

3.7. Effect of sleep deprivation on spatial learning

Studies on rodents suggest that the brain compensates for sleep *loss* by using preferably a striatal memory system (Hagewoud et al., 2010), further impaired hippocampal plasticity caused by sleep deprivation was associated with reduced use of allocentric strategy (Packard & McGaugh, 1996). Moreover, prolonged sleep fragmentation leads to reduced neurogenesis of the hippocampal dentate gyrus which promoted the use of a random, non-spatial search strategy (Sportiche et al., 2010). In another rodent study, sleep deprivation (SD) caused the increased activity of the hippocampal inhibitory neurons which can lead to impaired processing and consolidation of newly acquired spatial navigation consolidation (Delorme et al., 2021).

Total SD was shown to alert consolidation of spatial memories also in humans. One of the experiments was conducted in a real-life environment, i.e., a 1.2 km long route around an unfamiliar[39]

[38] Participants followed original routs instead of taking shortcuts. Taking shortcuts indicate flexible, allocentric representation of the encoded environment that is hippocampal-dependent.
[39] During the screening session, the participants were asked if they have even been in this neighbourhood.

neighbourhood in Rome (Ferrara et al., 2006). After learning the route, the participants performed a sequence-recognition task, where they have been asked to assess if presented snapshots of the route are displayed in the correct order. Next, the participants have been divided into three groups, where the *sleep* group was retested after one night of sleep, the *sleep deprivation* group after a night of total SD and the *wake* group after 8h of wakefulness. Participants across all groups demonstrated faster performance speed during the lab-based retest session, however, only the *sleep* group improved in the sequence-recognition task. The study was one of the first to support the idea that the newly learnt spatial memories seem to be enhanced by nocturnal sleep, whereas, SD tends to impair performance leading to a higher number of accuracy errors. No sleep EEG was recorded to capture potential sleep architectural changes induced by spatial learning. The following study conducted by the same group used a 3D virtual topographical orientation task and supported the view that sleep is critical for spatial memory formation and efficient usage of cognitive maps (Ferrara et al., 2008). Spatial navigational improvement was seen only when the encoding of the virtual environment was followed by nocturnal sleep. Again, the consolidation of spatial memories was altered by SD during the night following the encoding phase. The results are consistent with a recent study supporting the view that spatial location accuracy was improved after a night of sleep compared to a night of wakefulness, especially among participants with worse navigational baseline performance (Simon et al., 2021).

Interestingly, post-training sleep seems to also modulate preference regarding the spatial strategy required to navigate in the virtual environment (Orban et al., 2006). In the study, participants were divided into regular, nocturnal sleep or total SD group on the first night following a training session on the place-finding navigation task in a virtual city. Cerebral activity during the task performance was recorded using fMRI. During immediate (day 1) and delayed recall (day 4), enhanced activity was seen in the extended hippocampal network for both groups but *no* differences in the task performance were found. Interestingly, during a recall three days after the initial assessment (day 4), the individuals who were allowed to sleep during the first post-training night demonstrated a significant increase in striatal activity during recall of a virtual environment regardless of initial preference towards a hippocampus-dependent strategy. There was a linear relationship between striatal response and accuracy in the task and an association between hippocampus and striatum functional connectivity (FC) which shows that these associations were modulated by sleep during the night following the training. The study shows that post-training sleep helps to reorganize and shift the newly acquired memories to long-term storage, i.e., from hippocampus-based spatial navigational strategy to the striatum-based response strategy which suggests "*automation of the navigation behaviour*" (citation from Deantoni et al., 2021).

Consistently, Rauchs et al. (2008)[40] study also reported that post-training sleep promotes modulation of neuronal substrates associated with the consolidation of spatial and contextual memories encoded during spatial learning of a 3D virtual city. Notably, no behavioural changes in task performance were found neither in Orban et al. (2006) nor in Rauchs et al. (2008) studies. This suggests that one night of total SD might be not sufficient to impair the spatial performance on the given task.

A recent study conducted by Deantoni et al. (2021) using fMRI and an experimental design similar to Orban et al. (2006) (Figure 13) reported that navigation-associated activity continued during the resting state that followed directly the encoding of the virtual city. The activity was associated with changes in the FC, as well as, the amplitude of low frequencies in neural networks that were involved in task performance. Consistently, in another study, enhanced FC (increase in FC from pre- to post-initial learning[41]) between posterior hippocampus and dorsal caudate was shown in the *resting state* following a virtual water maze task which was associated with improved behavioural performance three days later (Woolley et al., 2015). This increase in water-maze-learning-specific FC was associated with an offline gain during subsequent task performance which supports the view that spatial navigation learning in humans is modulated also during post-task *awake* rest which emphasises how dynamic the integration and consolidation of spatial memories are and how complex those processes are from a temporal perspective.

[40] In Rauchs et al., 2008 study, the used navigation task was the same as in Orban et al., 2006, further, cerebral activity during task assessment was conducted using fMRI. The delayed retest took place in 72h after encoding. Participants were divided into sleep deprivation or full night sleep condition during the first night following the training session.

[41] Resting state activity was recorded during fMRI scan session for 7 minutes before and after task was performed, before each session, i.e., 4 scans in total. The participants were asked to fixate the gaze at the fixation cross, relax and do not think about anything specific.

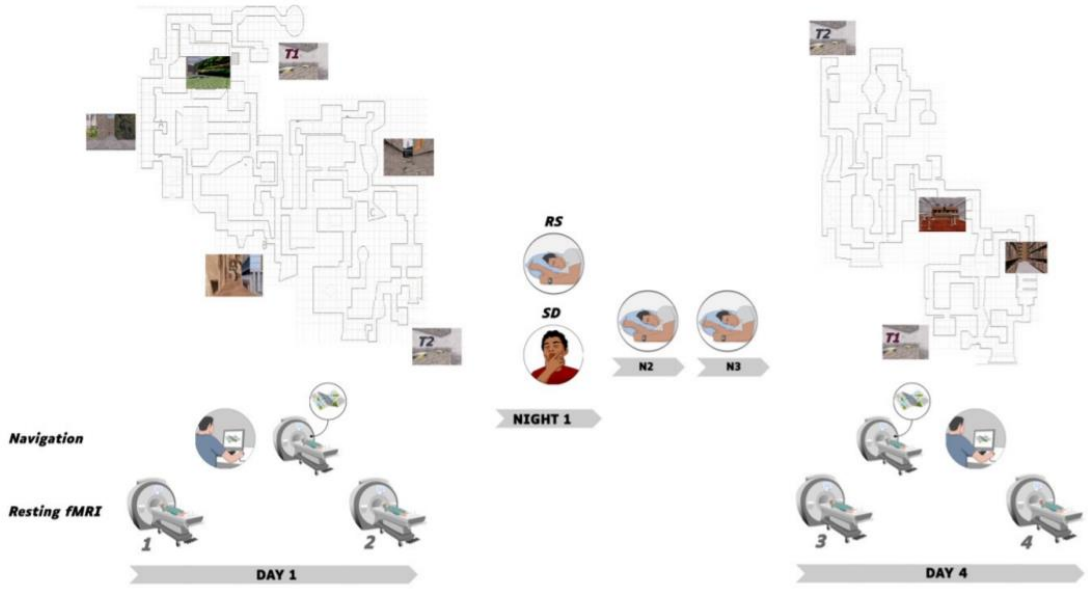


Figure 13. Experimental protocol used by Deantoni et al. (2021). Enhanced functional connectivity between navigation-specific brain areas in the participants in sleep deprivation who learnt an extended version of the virtual town during day 4 suggests that sleep deprivation leads to the necessity to involve more cognitive resources to associate new information (i.e., learning an extended version of the virtual town on day 4) with less efficiently memorized existing spatial memories (i.e., memory traces originated from learning the virtual task on day 1). Source of image Deantoni et al. (2021).

Conversely, post-training sleep but *not* wake was shown to lead to system-wide neuronal changes in humans and rats after allocentric and egocentric training (Samanta et al., 2020). The rats and the human participants were trained on either allocentric or egocentric conditions[42] of the water maze task and then further sub-divided into a post-training two-hours-long nap or two hours of wake while watching a movie while in rats 6h of SD or two hours of nap in rats. Post-training sleep was reported to lead to better memory performance in rats and humans when compared to wake conditions. Following sleep, in rats, recall-associated gene expression was seen in the hippocampus, striatum and prefrontal cortex regardless of the allocentric or egocentric training protocol. Notably, in the case of sleep-deprived rats, the recall-induced changes (seen as enhanced gene expression) were found in the striatum in the egocentric training group and the hippocampus in the allocentric one which demonstrates increased gene expression *only* in brain areas that are known to be needed for each task type. fMRI analysis in humans revealed significant changes (when compared to training sessions to the re-test) only in individuals who had taken a nap after the training. These neuronal changes were demonstrated in enhanced activation of superior posterior parietal cortices, and frontal medial cortex

[42] Human participants who were randomly allocated in the *allocentric condition* were given a different start location for each trail and needed to reorient themselves each time to find the target location, whereas in *the egocentric condition*, the start location for every trial was fixed, hence the participants needed to depend on repeated fixed movement while using landmarks to navigate towards the target location. In case of rats, in allocentric conditions, the animal was placed in a water maze at different starting quadrant for each task and needed to reorient itself to find a platform. In egocentric condition, the starting point was the same for each trail.

(linked to the executive control) and significantly reduced activity in the hippocampus, medial prefrontal cortex and precuneus (those structures are involved in the functioning of the default mode network). The outcomes of the study show that sleep promotes neuronal changes in allocentric and egocentric tasks with significant task performance gain as both rats and humans that slept performed significantly better compared to their sleep-deprived counterparts.

3.8. Impact of sleep manipulation and dreams on next-day spatial performance

Can we improve spatial navigation by manipulating sleep? The study using an EEG-based closed-loop targeted memory reactivation algorithm that was time-locked to the transition between the down-swing and up-swing of slow oscillations lead to significant improvement in navigation efficiency in a virtual city in young, healthy subjects (Shimizu et al., 2018). The study has shown that replaying sounds that were associated with learned spatial information (e.g., the sound of flowing water played when the subject was passing by the fountain, then the same sounds were replayed during the following nap) can improve the recall in a simple spatial navigational task (Figure 14). The participants from the closed-loop targeted memory stimulation group demonstrated greater improvement, i.e., more time-efficient navigation in comparison to the sham group. The study offers a promising way to enhance spatial navigation performance via sleep modulation.

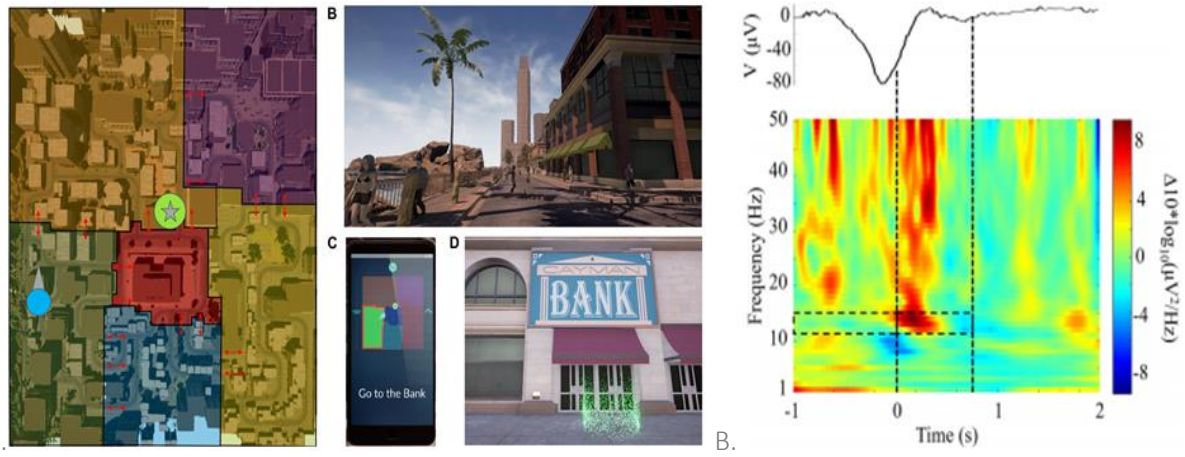


Figure 14. Enhancement of spatial navigation performance via sleep modulation. A. screenshot from the navigation task in virtual reality. The design of the environment was based on a large-scale, downtown-like area characterized by matched (congruent) sensory stimuli such as environmental sounds. The participants were instructed to learn how to navigate around the virtual city to reach specific landmarks. The algorithm run during the afternoon nap and aimed to evoke an increase in power in sleep spindle frequency spectral density (11-14 Hz). B. The time-frequency plot shows the difference between the mean response in the targeted memory reactivation group and the response at the same time point in control participants (sham condition) across the frontal electrodes Fp1 and Fp2. Figures reproduced from Shimizu et al. (2018).

Interestingly, incorporating elements of pre-sleep spatial learning into overnight dreams content was shown to be associated with enhanced memory consolidation and better virtual maze

navigation task performance[43] (faster maze competition time and shorter distance travelled) the next morning (Wamsley & Stickgold, 2019). The obtained results are in line with reactivation-based models of memory consolidation during sleep, where neuronal reactivation of recent experiences during sleep is associated with subsequent performance gains. Further, the study supports the idea that dreaming reflects memory processing.

3.9. Spatial performance and circadian rhythmicity

The study on elderly rhesus monkeys (19-25 years of age) who performed real-world spatial learning tasks demonstrated that daily *activity* levels were positively correlated with performance on the task, whereas decreased sleep quality was associated with worse spatial performance (Haley et al., 2009). The animals needed to retrieve a reward hidden in a single food port among 10 ports. Their rest-activity patterns and sleep data were recorded for 14 days using actigraphy (attached to the collar).

Further, chronic *jet lag*[44] was shown to lead to temporal lobe atrophy and spatial cognition alterations (Cho, 2001). Notably, frequent exposure to jet lag increases the level of the stress hormone – cortisol which prolonged secretion leads to the temporal lobe volume reduction. High levels of cortisol in elderly adults were shown to be associated with hippocampal atrophy and impairment of hippocampus-dependent learning and memory consolidation (Lupien et al., 1998). Further, rats kept under the chronic constant light condition that affected their circadian rhythmicity and induced stress (promoting increased cortisol realise) demonstrated impaired performance of the Morris water maze (Ma et al., 2007).

[43] The same task as in Nguyen et al., 2013 study.
[44] Jet lag is a *circadian rhythm* sleep-wake disorder caused by misalignment of the 24-hour internal clock and the new, local light and darkness patterns.

SUMMARY – APOE genotype, spatial navigation and sleep

Spatial navigation is a complex cognitive function involving highly specialized cells and widespread brain networks. The growing body of evidence shows that performance on spatial navigation tasks is influenced by APOE polymorphism with worse outcomes in ε4-allele carriers (e.g., Laczó et al., 2014, Nedelska et al., 2015, Coughlan et al., 2017). APOE genotype influences also the brain on a structural level (e.g., Konishi et al., 2016, Shaw et al., 2007, Heijer et al., 2002, Crivello et al., 2010). Furthermore, a significant overlap between the navigation system and brain regions affected by AD has been reported (Coughlan et al., 2018) and the studies revealed that spatial navigation performance shows high sensitivity to AD (Tu et al., 2015, Yew et al., 2013, Hornberger et al., 2010).

There is also a well-established bi-directional association between sleep and AD (Ju et al., 2013). However, it is not known whether early sleep problems may play any role in early changes in spatial navigation performance and if the potential associations are modulated by the APOE genotype. Importantly, it has been shown that closed-loop targeted memory reactivation during sleep and incorporating the spatial learning experience into dreams improved spatial navigation performance suggesting a mechanistic link between sleep and spatial learning (Shimizu et al., 2018, Wamsley & Stickgold, 2019). Remarkably, most of the cited studies investigating the impact of sleep on spatial learning involved mostly young adults which raises the question of what effect age has on observed associations, especially in the older population who are APOE-ε4 allele carriers.

Spatial navigational performance is underrepresented in sleep and circadian research. Consequently, it is not known how sleep pressure and time of day influence the performance of egocentric and allocentric spatial navigation tasks and if APOE- ε4 allele carriership modulates potential associations. This in turn highlights the research gap which the presented PhD project aims to address.

Chapter IV - Objectives and Research Questions

4.1. Objectives

Objective 1: to investigate the impact of the APOE-ε4 allele on sleep, circadian rest-activity patterns and cognition

Objective 2: to study the impact of APOE-ε4 allele carriership on the relationship between sleep, circadian rest-activity patterns and spatial navigation

Objective 3: to examine the effects of homeostatic sleep pressure and time of the day (i.e. circadian phase) on allocentric and egocentric spatial navigation and their modulation by APOE-ε4 allele carriership

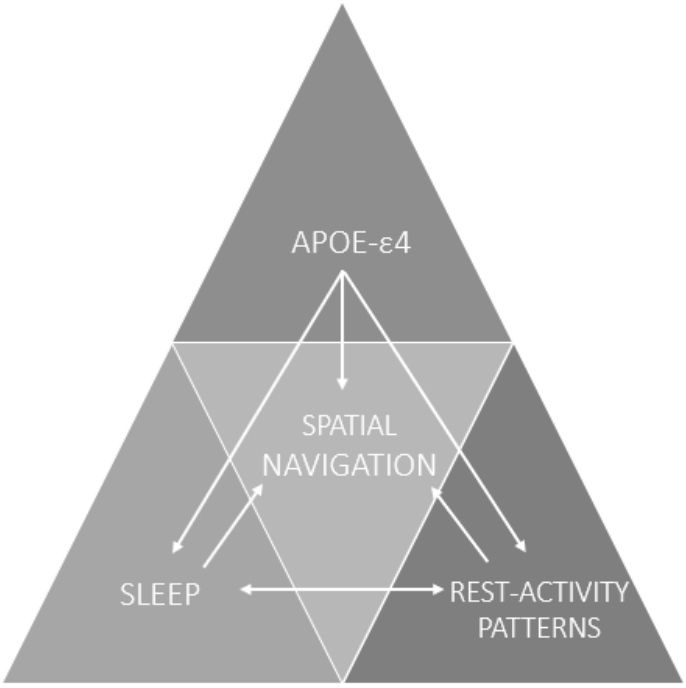


Figure 15. The graphical representation of the investigated interactions.

4.2. Research Questions

The analyses presented in the PhD thesis followed strictly research questions that were established early on in the PhD project. Hence the analyses were mostly explorative.

4.2.1. Research Questions - Screening session *(see Methods)*

1. Does APOE-ε4 allele carriership affect self-reported sleep, chronotype, and cognition independently of potential confounding factors such as age, and biological sex in healthy elderly adults?

2. What is the association between spatial navigation, self-reported sleep, chronotype and cognition in healthy elderly adults?

3. Does the APOE-ε4 allele carriership modulate associations between self-reported sleep, chronotype and spatial navigation in healthy elderly adults?

4.2.2. Research Questions - Actigraphy (Field) session *(see Methods)*

1. Does the APOE-ε4 allele affect actigraphy-assessed circadian rest-activity patterns in the habitual environment regardless of possible confounding factors such as age and biological sex?

2. What is the relationship between the actigraphy-assessed circadian rest-activity rhythmicity in the habitual environment and spatial navigation and how is the association modulated by the APOE-ε4 allele carriership?

3. Does the APOE-ε4 allele affect self-reported sleep quality and sleep efficiency as measured by sleep diary in the habitual environment regardless of possible confounding factors such as age and biological sex?

4. What is the association of subjective sleep quality as measured by sleep diary in the habitual environment with spatial navigation and how is the association modulated by the APOE-ε4 allele carriership?

4.2.3. Research Questions - Lab session *(see Methods)*

1. To what extent are objectively and subjectively measured vigilance, working memory, episodic memory performance and subjective mental effort affected by low and high sleep pressure conditions and time of day (i.e. circadian phase) in healthy elderly men and women?

2. What is the effect of sleep pressure and time of the day on allocentric and egocentric navigation?

3. Does the genetic risk of AD (APOE-ε4 allele carriership) modulate the effect of sleep loss and time of the day on objectively and subjectively measured vigilance, working memory, episodic memory and subjective mental effort?

4. Does the genetic risk of AD (APOE-ε4 allele carriership) modulate the effect of sleep loss and time of the day on spatial navigation?

5. Does objectively measured baseline sleep architecture differ between the low (APOE ε4 allele non-carriers) and high (ε4 allele carriers) genetic risk of AD in healthy elderly?

6. Does the genetic risk of AD (APOE-ε4 allele carriership) affect sleep propensity and sleep architecture across consecutive naps?

7. Does the APOE-ε4 allele modulate the homeostatic response of sleep duration and architecture to sleep loss (comparison of sleep architecture parameters between Baseline and Recovery Nights)?

CHAPTER V – METHODS

5.1. Ethics

The study "*Early sleep and circadian markers of Alzheimer's disease: The impact of APOE-ε4 on circadian rhythm and sleep-wake homeostasis in humans*" was approved by the Faculty of Medicine and Health Sciences Research Committee of the University of East Anglia. The study was compliant with General Data Protection Regulation and the Data Protection Act 2018.

5.2. Compensation and Reimbursement of Research Participants

The participants were reimbursed for all the study-related travel expenses including the parking space needed for the duration of the screening sessions. Further, the participants were paid £50 for the field session and £250 for the sleep laboratory session. Importantly, the payment was rather a compensation for participants' time than the main reason for individuals to consider participation in the study.

5.3. Study design

The study involved *observational* and *experimental* methods (Figure 16). The observational study investigated self-reported and objectively assessed features of sleep, rest-activity patterns, chronotype, cognition and general physical and psychological health (i.e., Screening session and Field session). The experimental part aimed to establish the causal effects of sleep restriction and time of the day (circadian phase) on self-reported well-being, cognitive performance, sleep micro-and macroarchitecture and wake brain activity (Laboratory session). In both cases, the effects of the APOE genotype were assessed.

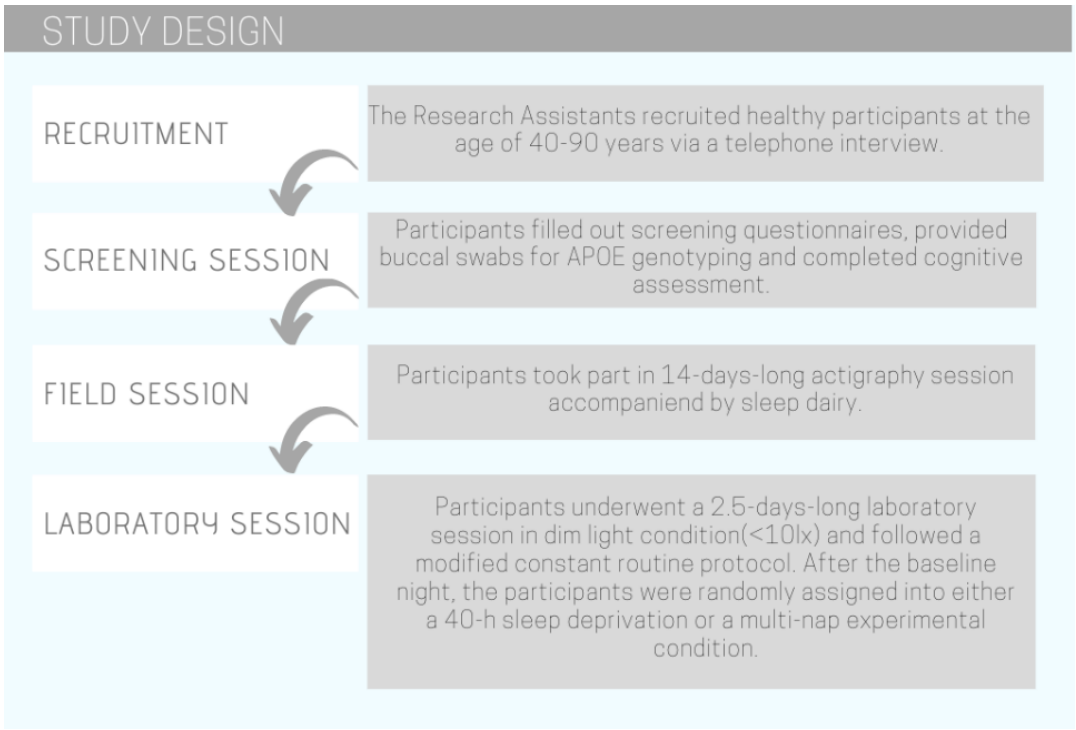


Figure 16. Study design. The study design consisted of four stages, recruitment, screening session, field session (home-based session) and laboratory session.

5.4. Recruitment

The participants were recruited via various channels, i.e., Join Dementia Research, University of East Anglia APOE-ε participants database, University of the Third Age and advertisements. The Sleep and Brain Research team recruited healthy participants at the age of 40-90 years via a telephone interview making sure that any of the following *initial* exclusion criteria are met:

- pregnancy
- acute infections or diseases
- psychiatric disorders or chronic neurological conditions including a diagnosis of dementia, mild cognitive impairment (MCI), prodromal AD
- learning, sensory or physical impairment and/or disabilities that would pose an unfair disadvantage in the cognitive tests
- chronic pain conditions
- a current diagnosis of malignant tumours/cancers
- a shift work
- consuming more than 14 units of alcohol a week and/or use of illicit substances/alcohol dependency
- smoking tobacco, e-cigarettes or vapes

5.5. Screening Process

Individuals who met *initial* eligibility criteria were invited for a screening session at the Sleep and Brain Research Unit (SBRU), based at the University of East Anglia, Norwich, England. At the beginning of the screening session, a short presentation describing the stages of the study was given and participants had an opportunity to ask questions before signing the informed consent[45]. Then, the subjects were asked to fill out screening questionnaires (40-60 minutes), complete the cognitive assessment (45-60 minutes) and provide two buccal swabs for DNA extraction and subsequent APOE genotyping. The questionnaire session and cognitive assessment session were conducted in blocks and their order was assigned according to the wish of the subject (see Appendix 3 - Screening protocol). The buccal swabs were taken between two sessions during a short 10-minutes-long break[46].

5.5.1. Questionnaires

The questionnaires were divided into *priority* questionnaires that needed to be completed during the screening session (prior Covid-19 pandemic, see footnote[2]) and *non-priority* questionnaires that could be completed at home and sent back to the study team in a pre-paid envelope. In the presented PhD thesis, the analysis focused on priority questionnaires only (Table 4) as they provide a comprehensive picture of sleep quality, rest-activity patterns, subjective cognitive decline assessment and mental health.

Non-priority questionnaires included the following forms: Edinburgh Handedness Inventory (Oldfield, 1971), the Dimensional Apathy Scale (Radakovic & Abrahams, 2014), Empathy Quotient (Baron-Cohen & Wheelwright, 2004), the Barratt Impulsivity Scale (Patton et al., 1995), Big Five Inventor (McCrae & Costa, 1987), Positive & Negative Affect Scale (Watson et al., 1988), Short-form Health Survey (Ware & Sherbourne, 1992), Dutch Eating Behaviour Questionnaire (van Strien et al., 1986), the Mannheim Dream Questionnaire (Schredl et al., 2014).

[45] The informed consent was sent to each participant at least 48h before the screening session. During the screening session, two witnesses were present while the participant was signing the consent.

[46] During the period of the Covid-19 pandemic, 10-minutes-long break was skipped. To further minimalize time of close contact with the participants, we used online-based versions of the questionnaires (implemented in Microsoft Forms) and/or paper-based forms which could be filled out at home and send to the study team using pre-paid envelopes. Consequently, the participants were coming to the University of East Anglia campus only for the cognitive assessment and the buccal swab.

Questionnaire [range of scores]	Description
• Demographics Questionnaire[47] • General Medical Questionnaire[48]	• basic demographics • the detailed medical assessment was evaluated by a medical doctor before the sleep lab session
• Epworth Sleepiness Scale (ESS) [0-24] (Johns, 1991) • Pittsburgh Sleep Quality Index (PSQI) [0-21] (Buysse et al., 1989) • the Insomnia Severity Index (ISI) [0-28] (Bastien et al., 2001)	• a measure of daytime sleepiness • assessment of sleep quality over the last month • evaluation of the severity of perceived nocturnal and diurnal symptoms of insomnia over the last two weeks
• Morningness- eveningness questionnaire (MEQ) [16-86] (Horne & Östberg, 1976) • Munich Chronotype Questionnaire (MCTQ) [16-86] (Roenneberg et al., 2003)	• assessment of diurnal preference and chronotype
• Patient Health Questionnaire (PHQ-9) [0-27] (Kroenke et al., 2001) • Generalized Anxiety Disorder Questionnaire (GAD-7) [0-21] (Spitzer et al., 2006)	• assessment of depressive symptoms and their severity over the last two weeks • a measure of initial symptoms and severity of anxiety over the last two weeks
• Cognitive Change Index (CCI) [20-100] (Rattanabannakit et al., 2016) - CCI memory [12-60] - CCI executive functions [5-25] - CCI language [3-15]	• a questionnaire measuring the current subjective cognitive decline in memory, executive function and language domains relative to 5 years ago

Table 4. Priority questionnaires and their descriptions.

5.5.2. Cognitive assessment – screening session

Currently, the diagnosis of Alzheimer's disease is based on the history of worsening amnestic and non-amnestic symptoms in the visuospatial, language and executive functions (Dubois et al., 2014). Hence, during the screening session, we implemented a cognitive test battery assessing several cognitive functions (Table 5). A Mini-Addenbrooke's Cognitive Examination task (m-ACE) version B was

[47] In the demographics questionnaires, the participants were asked to provide their date of birth, biological sex, years of education, highest obtained level of education, yearly income, hight and weight.
[48] General Medical Questionnaire covered questions related to health, lifestyle, medication and GP address. The questionnaire included also selected physical activity questions from the Elderly International Physical Activity Questionnaire (Hurtig-Wennlf et al., 2010).

used to screen for dementia and Mild Cognitive Impairment (Hsieh et al., 2015). Karolinska Sleepiness Scale (KSS; Akerstedt & Gillberg, 1990) was administrated before and after cognitive assessment to measure subjective sleepiness. Sleep quality and quantity for the previous night were assessed using the Karolinska Sleep Diary (KSD; Akerstedt et al., 1994). Importantly, the outcomes of the administrated cognitive battery can serve as baseline measures for a potential longitudinal cohort study.

Outcome measures – cognitive assessment – screening session

Task *task length* [range of scores]	Assessed Cognitive functions	Outcome measures
mini Addenbrooke's Cognitive Examination (m-ACE; Hsieh et al., 2015) *task length*: ~5 min [0-30]	global cognition is assessed across five domains: attention, memory, verbal fluency, language and visuospatial abilities; m-ACE is a dementia screening tool	- total score
Symbol Digit Modalities Test (SDMT; Smith, 1982) *task length*: ~3-4 min [0-110]	attention, executive functions, and speed of processing; SDMT is a sensitive tool to detect cognitive impairment	- the number of correct substitutions in a 90-second interval
Trail Making Tests Part A and B (TMT; Reitan, 1992) *task length*: ~3-4 min [errors Part A: 0-22] [errors Part B: 0-24]	attention, visuospatial attention, psychomotor speed	- time needed to complete Part A and Part B (sec) - number of errors made in Part A and Part B
Rey Complex Figure Test[49] (ROCF; Meyers & Meyers, 1995) *task length*: ~3-5 min for each element [raw score for copy and recalls: 0-36]	visuospatial abilities, memory, attention, planning, and working memory	- Raw score copy - Raw score immediate recall - Raw score delayed recall - the time needed to complete each trial (sec) - Recognition task [50]
Hopkins Verbal Learning Test-Revised[51] (HVLT-R; Benedict et al., 1998) *task length*: ~2 min for each element [total recall: 0-36] [delayed recall: 0-12]	verbal memory	- Total recall[52] - Delayed recall (i.e., Trial 4) - Retention[53] - Recognition Discrimination Index[54]

[49] Assessment included: copy, immediate recall (3 min after the copy trail) and delayed recall (30 min after the copy trail) which was immediately followed by the recognition task.
[50] Recognition = number of true positives – number of false positives
[51] Assessment included: encoding – 3 trails, delayed recall (administered 20-25 min after the 3rd encoding trail) and the recognition task.
[52] Total recall = sum of total correct responses for Trials 1,2 and 3
[53] Retention (%) = [(Trial 4 / Higher score of Trials 2 and 3)*100]
[54] Recognition Discrimination Index = Total number of true positives – Total number of false positives

[recognition score: 0-12]		
Supermarket Task (Tu et al., 2017) *task length*: ~10-15 min [egocentric score: 0-7] [head direction score: 0-7]	spatial navigation ▪ egocentric ▪ allocentric	egocentric component: - a number of responses indicating the correct location of the starting point allocentric component: - the correctness of the marked final location heading direction: - number of correct judgments of a final heading direction

Table 5. Cognitive tests administrated during screening sessions with corresponding variables of interest.

5.5.3. The measure of spatial navigation – Supermarket task

In addition to the well-established cognitive tests, the iPad-based Supermarket task (Tu et al., 2017) was administered to measure spatial navigation. The Supermarket task consists of *allocentric* and *egocentric* components. In the egocentric trials, the participants watched a video of a route taken across the virtual supermarket's alleys (Figure 17A). The 20 seconds-long video was displayed from the first-person perspective and included two turns. At the end of each trial, i.e., when the video stopped, the participants were asked to point where the starting point is located, i.e., in the front or behind, on the left or the right (Figure 17B). The participants were instructed to provide as precise answers as possible while imagining that the range of potential answers was equal to 360°. All trails began at the same location but followed different routes and had different endpoints.

Each egocentric trial was followed by an allocentric task, where the participant was presented with a map of a supermarket and asked to point out the location where the video stopped (Figure 17C) and to specify the final heading direction of the trolley, i.e., North, South, West or East (Figure 17D). The task consisted of seven trials preceded by two to five practice sessions (i.e., if the participant has not completed successfully 1/2 of the practice trials, the next practice opportunity, including three trials, was given). In each practice trial, the same training video was presented and feedback was provided, however, no feedback was given for the assessment trials. The supermarket displayed in the task was created in an ecologically valid manner and without any notable landmarks which could be encoded and facilitate memorisation of the path.

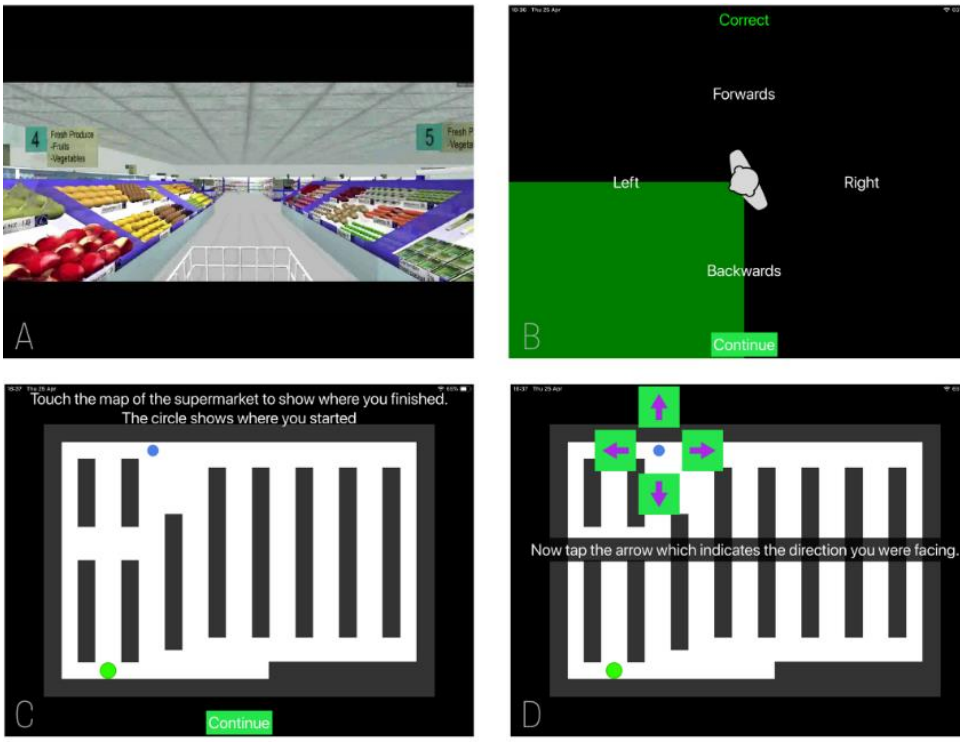


Figure 17. The Supermarket task. Participants watched a video of a shopping trolley moving around the supermarket (A) Once the video stopped the participant was asked where is the starting point, i.e., relative to where the trolley has stopped (B), then to mark on the map where the trolley stopped (C) and in which direction was facing (D). Screenshot B shows an egocentric component of the task, whereas C and D are the allocentric ones. The presented images are screenshots of the Virtual Supermarket Task (Tu et al., 2017).

5.5.4. APOE genotyping

Two buccal swabs (Sigma Dry Swab Tubed, MW941) were taken during the screening session. To avoid potential contamination, the participants were asked to not consume any food or drink 30 minutes before swab collection. The participants were instructed to rotate and rub the swab against the inside of a cheek for 30 seconds while avoiding touching teeth and tongue. Then, right after the collection, the ID-labelled samples were stored in the SBRU fridge (~3°C). DNA was extracted within 5 days at the Medical Research Laboratory at the Bob Champion Research and Education Building, at the University of East Anglia.

DNA extraction was done using the QIAamp® DNA Mini Kit following the manufacturer protocol "DNA Purification from Buccal Swabs". Extracted DNA was then spectrophotometrically quantified using NanoDrop™ software. The outcomes of interest were Nucleic Acid Concentration, 260/280 ratio and 260/230 ratio. Ratio 260/280 measures the wavelength of Nucleic Acid/Protein, the DNA with a ratio value between 1.8 and 2.0[55] is considered "pure" and a peak of absorbance at about 260 nm is typical

[55] Higher value indicates that the sample contains high concentration of proteins which are not DNA, whereas lower value will indicate low concentration of DNA in the probe.

for DNA. Ratio 260/230 is a secondary measure of nucleic acid purity and measures Nucleic Acid/Organic compounds ratio with a recommended value between 1.8 and 2.2[56]. The ID-labelled Eppendorf tubes with extracted DNA were stored in the freezers (-80°C) at the Bob Champion Research and Education Building until a sufficient number of samples was collected to run a quantitative polymerase chain reaction (qPCR) using the TaqMan probe.

The Real-time PCR APOE SNP genotyping assay was done following a well-established laboratory protocol provided by Professor Anne-Marie Minihane's group (see Appendix 4 - qPCR plate preparations' protocol, the same protocol was used by Coughlan et al. (2019). Samples with a dilution ratio >1 were diluted using Nuclease-Free water. The dilution ratio was calculated based on the results obtained via DNA quantification using NanoDrop™ software (Table 6).

DNA concentration [ng/µl]	Dilution ratio	Sample [µl]	Water [µl]	Total volume [µl]	DNA concentration after diluting [ng/µl]
30.8	2	5	5	10	15.4
5.9	1	5	0	5	5.9

Table 6. An example of dilution ratio calculations. For the APOE genotyping, each sample needed to contain from 1 to 20ng/µl of purified genomic DNA. If it was not the case, the sample was diluted with Nuclease-Free water. Hence, if the concentration of a sample was, for example, 30.8ng/µl, then 5µl of water was added to 5µl of the DNA sample to dilute it to the concentration <20 ng/µl. After the dilution with 5µl of water, the DNA concentration = 15.4ng/µl (30.8 ng/µl / 2 (a dilution ratio)).

Next, a 96-well reaction plate set-up map indicated the precise location of each sample, i.e., participants' DNA samples, three positive controls and three negative controls on sides 112 and 158 (see Appendix 5 – Exemplary qPCR plate set-up). Each well contained 10µL of the following components: 8µL of TaqMan Genotyping Master Mix (TaqMan Genotyping Master Mix, 20x working stock of SNP Genotyping Assay (112 or 158) and RNase and DNase free water) and 2µL DNA sample (containing 1-20 ng purified genomic DNA).

The qPCR run followed the same steps as in Coughlan et al. (2019) (see Appendix 6 - qPCR machine set-up). All genotypings were run on the same machine - Applied Biosystems 7500 Real-Time PCR System (96-well format) located in the Biomedical Research Centre, at the University of East Anglia, using the Applied Biosystems 7500 Real-Time PCR System. The genotypes were determined based on the reference table (Table 7). After successful genotyping (13 runs in total), the samples with extracted DNA and the participant's ID were moved to long-term storage at the Norwich Research Park Biorepository, Norwich, England.

[56] If the value is higher, it means that the sample is contaminated; if the value is lower, the amount of DNA is low.

APOE genotype	112	158
ε3/ε4	CT (1/2)	CC (1/1)
ε2/ε2	TT (2/2)	TT (2/2)
ε2/ε3	TT (2/2)	CT (1/2)
ε2/ε4	CT (1/2)	CT (1/2)
ε3/ε3	TT (2/2)	CC (1/1)
ε4/ε4	CC (1/1)	CC (1/1)

Table 7. APOE genotyping reference table. Three APOE isoforms differ by a single amino acid, i.e., arginyl (Arg) and cysteinyl (Cys) at positions 112 and 158, where, ε2: Cys112, Cys158, ε3: Cys112, Arg158 and ε4: Arg112, Arg158 (Zhong& Weisgraber, 2009). There are six combinations of the codominant alleles linked to two single-nucleotide polymorphisms, i.e., ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4.

5.6. Field session

5.6.1. Eligibility criteria – field and sleep laboratory sessions

To take part in the field and/or sleep laboratory sessions the participants needed to meet the following eligibility criteria PHQ-9 $\leq$ 9; GAD-7 $\leq$ 9; ISI $\leq$ 14; ESS $\leq$ 10; m-ACE $\geq$ 25 and being a carrier of ε3/ε3, ε3/ε4 or ε4/ε4 APOE genotype. All ε2 allele carriers were excluded because of the inability to obtain a representative sub-group due to the small percentage of the ε2 allele carriers in the European-ancestry population (Kuo et al., 2020). Further, to take part in the sleep lab session participants needed to successfully pass a medical examination conducted by a physician.

5.6.2 Data collection

Following the screening session, eligible participants were invited to take part in a two-week-long home-based field session aiming to assess their habitual rest-activity patterns. The data collection included a continuous actigraphy recording and a sleep diary. Actigraphy and sleep diary were sent to the participants by post upon agreeing on the most convenient time for the session and making sure that the recording will cover habitual activity and not, for example, vacation where travelling and holidays activity can alter typical wake-activity patterns. We have also made sure that the participants have not travelled through several time zones three months before the session. If the participant was interested only in the field session (and not in the laboratory-based session), then the actigraphy package included also a payment form and another pre-paid envelope so the participant could ask for reimbursement for postage of the sleep diary and actiwatch and be paid for the participation. In the provided instructions, participants were asked to send the actiwatch and sleep diary in one envelope and the reimbursement form in a separate one to avoid sending the participant's unique study code (listed on the sleep diary) and his/hers name (provided on the bank details) in one package.

Throughout the field session, the participants were asked to wear an actiwatch (MotionWatch 8, CamNtech©) on their non-dominant wrist and avoid taking it off as much as possible[57]. The actiwatch is a watch-sized, light-sensitive, waterproof device equipped with a small accelerometer that records gross motor activity providing information about rest-activity cycles (Figure 18). Besides the accelerometer, the actiwatch contains also a real-time clock, low-pass filter (to remove irrelevant external vibrations that are not associated with the gross motor activity), memory storage, light exposure sensor and USB port (the MotionWear User Guide). In this project, to obtain a high resolution of the recording, the epoch length was set to 15 sec.

Additionally, the participants were asked to complete a sleep diary twice a day, i.e., right after getting up and before nocturnal sleep. The diary consisted of Karolinska Drowsiness Task (KDT), KSS, selected items from the Mannheim Dream questionnaire (MADRE), Elderly International Physical Activity Questionnaire, visual analogue scales (VAS) measuring mood as well as questions about diet, light exposure and physical activity (see Appendix 7 - Sleep diary). The participants received two sleep diary booklets, i.e., week 1 – days 1-7 and week 2 – days 8-14 including detailed instructions. A summary of analysed outcome measures can be found in Table 9.

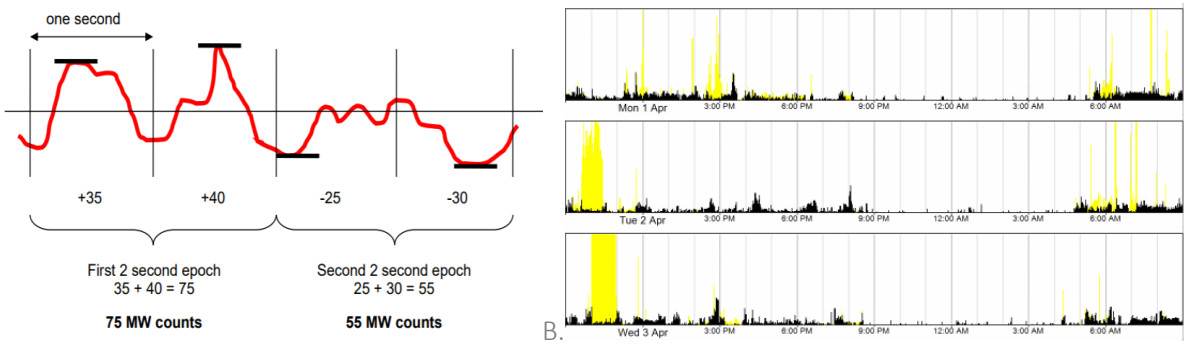


Figure 18. Acquisition of actigraphy data. Graph A. shows how the accelerometer data are processed and recorded, i.e., a bandwidth filter is used to discard the frequencies from 3-13 Hz. Then either a positive or negative peak is recorded per second and the value is compared to a minimum, i.e., the 'not moving' threshold (~0.1g). If the value is below the threshold, it is omitted. Then, the value of each second is summed over an epoch giving a value for each epoch. B. shows three days of 24h actogram where each row indicates one day and one night. Each day and night consists of 5760 epochs (15 sec per epoch = 4 epochs per minute). The black spikes indicate the activity levels, i.e., the summed gross motor activity. One can see wake when the activity is present for a prolonged time, whereas sleep when the activity is significantly reduced (e.g., from 9 PM to 6 AM there are only isolated spikes of activity suggesting movement during sleep and/or arousals). It is important to bear in mind that when a participant sits still, e.g., reading a book might look like a sleep episode (a nap), therefore, it is always important to cross-reference the actigraphy with sleep diary entries. Further, the yellow highlight shows light intensity. Highlights with a high amplitude suggest very bright light exposure, for example, while working in the garden. Image source: MotionWear User Guide

[57] All actiwatches were cleaned and disinfected before sending. During the Covid-19 pandemic, we have used a professional spray killing bacterial and viruses including Covid-19. Prior the pandemic, we have used alcohol wipes.

5.6.3. Data analysis

Actigraphy data were analysed using the pyActigraphy Python package (Hammad et al., 2020). Each data file was cleaned separately to discard periods of unintended locomotor inactivity, such as breaks in wearing an actiwatch (defined as lack of signal ≥2h) and periods before and after the scheduled time of data collection[58]. Only well-established rest-activity variables such as Non-Parametric Circadian Rhythm Analysis, Cosinor analysis and State Transition analysis were analysed because of evidence that using actiwatch has alarmingly low accuracy of sleep staging and quantifying sleep (Ameen et al., 2019), especially when the ECG signal is not recorded.

Non-Parametric Circadian Rhythm Analysis involved Interdaily Stability (IS), Intra-Daily Variability (IV) and Relative Amplitude (RA) (van Someren et al., 1999). Parametric analyses included State Transition analysis (Lim et al., 2011) and Cosinor analysis (Refinetti et al., 2007). The State Transition Analysis is based on the probabilistic state transition model quantifying the fragmentation of human rest-activity patterns providing matrices of Activity-to-Rest probability (kAR) and Rest-to-Activity probability (kRA). Higher values indicate a higher probability of the transitions between the states of rest and activity.

Cosinor analysis aimed to estimate the parameters of the actigraphy time series by fitting a cosine curve with a period of 24 h (Figure 19). Fitting the curve allows deriving estimates of key parameters of the circadian rest-activity rhythmicity such as Midline Statistic Of Rhythm (MESOR), Amplitude and Acrophase. For the analysis, the period was fixed to 5760 for data with a 15sec sampling rate and 2880 with a 30sec (if the actiwatch was set to a 30-sec epoch by mistake). The F statistic was used to indicate how well the data match the cosine function, i.e., the goodness of fit to curve, and measure of robustness. A summary of analysed outcome measures can be found in Table 8.

[58] The actiwatches were sent to the participants by post and if the delay in dispatch occurred, the recording could have started before the delivery what produced unwanted data. Further, once the period of two weeks of wearing the actiwatch passed the participants have been asked to send the actiwatches back to the SBRU (if sleep lab session has not followed) what led to the situation where the data were recorded during delivery creating again invalid data points.

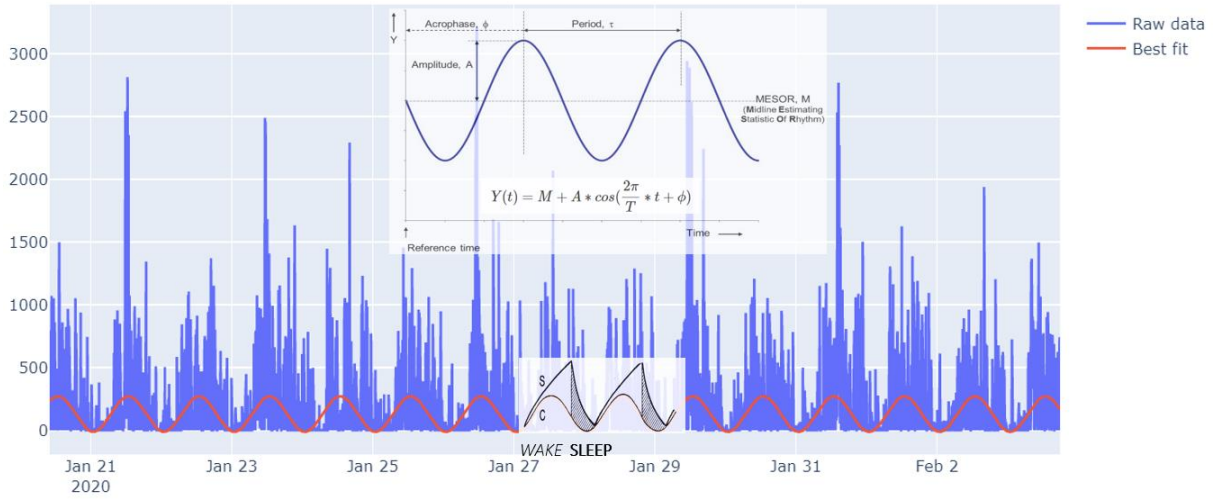


Figure 19. An example of the Cosinor Analysis outcome plot of a single participant was generated using the pyActigraphy (background plot). The red sinusoid shows process C, indicating activity (higher activity levels, i.e., higher and more dense spikes) and rest (lower activity, i.e., lower amplitude and a smaller concentration of spikes). The graph on the top describes the Cosinor Analysis, where M is the MESOR, A is the amplitude of the oscillations, T is the period and ϕ is the acrophase. Image: Cornelissen (2014), formula: Hammad et al. (2020)

5.6.4. Outcome measures – actigraphy

	Variables [score range]	Description
Non-Parametric Circadian Rhythm Analysis	Interdaily Stability (IS) [0-1]	Indicates the degree of stability in the activity-rest pattern (day-to-day consistency). The variable has a range of 0 to 1 where 0 is a total lack of rhythm and 1 is a completely stable rhythm.
	Intra-Daily Variability (IV) [0-2]	Provides the degree of fragmentation of the activity-rest periods with a range from 0 to 2, where higher values signalling higher fragmentation. Typical values for the healthy participant will be below 1.
	Relative Amplitude (RA) [0-1]	RA = the mean activity level during the 10 most active hours (M10)[59] – the mean inactivity in the 5 least active hours (L5)[60]. RA ranges from 0 to 1 with higher values showing a rhythm with higher amplitude.
State Transition Analysis	Activity-to-Rest probability ($kAR_{probability}$) [0-1]	The higher the kAR, the poorer sustained periods of activity are, while the lower the kAR indicates a higher consolidated.
	Rest-to-Activity probability ($kRA_{probability}$) [0-1]	The higher the kRA, the more fragmented the periods of rest are, while the lower the kRA, the more sustained they are.

[59] M10 (10 most active hours) Average - Indicates the average activity level for the cycle of ten most active hours and is an indicator of how active and regular the wake/active periods are.
[60] L5 (5 least active hours) - Indicates the average activity level for the cycle of five least active hours and is an indicator of how inactive and regular rest/sleep periods are.

Cosinor Analysis	MESOR	Midline Statistic Of Rhythm (MESOR) is the rhythm-adjusted mean that is the average value of the cosine curve fitted to the data
	Amplitude	The difference between the maximum and the MESOR.
	Acrophase	Timing of highest activity.

Table 8. Rest-activity variables of interest. Circadian variables are grouped by analysis method nonparametric, parametric State Transition and Cosinor.

5.6.5. Outcome measures – sleep diary

Sleep feature	Description
Total time in bed (TIB)	time from lights out to wake-up time [hours] [minutes]
the actual number of hours asleep	TIB – (sleep latency + time awake)
Sleep efficiency (SE)	(Actual hours of sleep/TIB)*100 [%]
Subjective sleep quality (SQ)	the participant was asked to indicate sleep quality upon morning's awakening following the KSS scale, ranging from 1 to 9, where 1 indicates "best sleep ever" and 9 is "worse sleep ever"
Midpoint of sleep	clock time corresponding to half the of the sleep period time

Table 9. Sleep diary – variables of interest. The variables of interest included only sleep-related information which was reported by the participants after each sleep episode over 14 days.

5.7. Laboratory sessions

5.7.1. Data collection

Following the field session, the same eligible individuals were invited to take part in the laboratory session. During the laboratory sessions, participants were residents in the SBRU[61] for a total

[61] The SBRU sleep unit consists of one bedroom with ensuite bathroom and monitoring room. The bedroom was equipped in single bed, bedside table, two desks with two computers (i.e., two monitors, two PC, two keyboards, two mouse), TV, closet, armchair, coffee table, two neutral paintings, blackout window, curtain, a pedalling device, force plate (balance test) camera

of approximately 2.5 days including three consecutive nights. The laboratory session consisted of two experimental protocols aimed to modulate sleep pressure, i.e., 40h of total sleep deprivation vs. 40h of multinap protocol (Figure 20). These types of protocols are commonly used to challenge sleep-wake homeostasis allowing for assessing the effect of high vs. low sleep pressure on brain activity, cognitive performance, the emotional state during wakefulness and subsequent recovery sleep (e.g., Graw et al., 2004, Cajochen et al., 2001).

At the beginning of each sleep laboratory session, the participants had time to unpack, get used to the environment and familiarize themselves with the unit. Then, a medical doctor reviewed the participant's General Medical Questionnaire and performed a physical examination to assess medical eligibility to take part in the laboratory session. After the medical check, dinner of the participant's choice was provided. After dinner, participants have taken part in the practice trial involving all the cognitive tasks (COGT). Usually, after the COGT practice, the participants had a bit of free time and could watch Netflix, read a book or talk with the study team. Then, both of the protocols started with the baseline sleep episode involving polysomnography (PSG) with an extended clinical montage to screen for potential primary sleep disorders (such as Obstructive Sleep Apnoea, REM Sleep Behaviours Disorder, NREM sleep parasomnias, Periodic Limb Movement Disorder). The timing of the baseline and recovery nights followed the habitual sleep timing of each participant as measured during the field session (i.e. actigraphy/sleep diary). Participants were asked to follow regular sleep and wake-up schedule three days before the lab session. The baseline PSG recordings were monitored by the Principal Investigator of the project to screen for sleep disorders. The protocols finished with the recovery night where the participants had an 8-12h-long sleep opportunity ad libitum.

Both experimental protocols included the cognitive tests battery (see Cognitive assessment – sleep lab session) that was assessed on a 4-hourly basis along with EEG recording and followed by a Static Posturography task (i.e., balance test). To measure the phase and the amplitude of the intrinsic circadian clock, melatonin concentration was repeatedly sampled between the 9th and 31st hours of the protocols (see Outcome measures - melatonin). Throughout the extended wake period, the light was kept at a dim level (< 10lux) to avoid the impact of high-intensity light on melatonin production and level of alertness as well as to eliminate the light-related time cue.

During the protocol, in their *free time*, participants could watch Netflix[62], read books, and magazines, do jigsaw puzzles, walk around the bedroom, use a pedalling device or talk with the experimenters. The participants received regular snacks every 90 minutes. Each snack was limited to

and microphone to communicate with the study team. The bathroom consisted of shower, sink, toilet and mirror. All the lights in the unit were dim. The participant was not allowed to enter the monitoring room.

[62] The study team was asking to avoid watching anything what can affect the mood or/and well-being of the participant.

150Kcal for females and 200Kcal for males and could include grapes, cocktail tomatoes, cucumber, baby carrots, bell peppers, cheese and bread. Water was provided at all times ad libitum, whereas fruit teas (caffeine-free) were offered only at snack times. Only one participant at the time was taking part in the laboratory session.

In the morning following the recovery night, the participants could take part in an optional, additional cognitive assessment. The supplementary assessment consisted of Raven Progressive Matrices (Court & Raven, 1998), Digit Span task (Kaufman & Lichtenberger, 1999) and Reward prediction error test (Sambrook & Goslin 2014, 2016)[63].

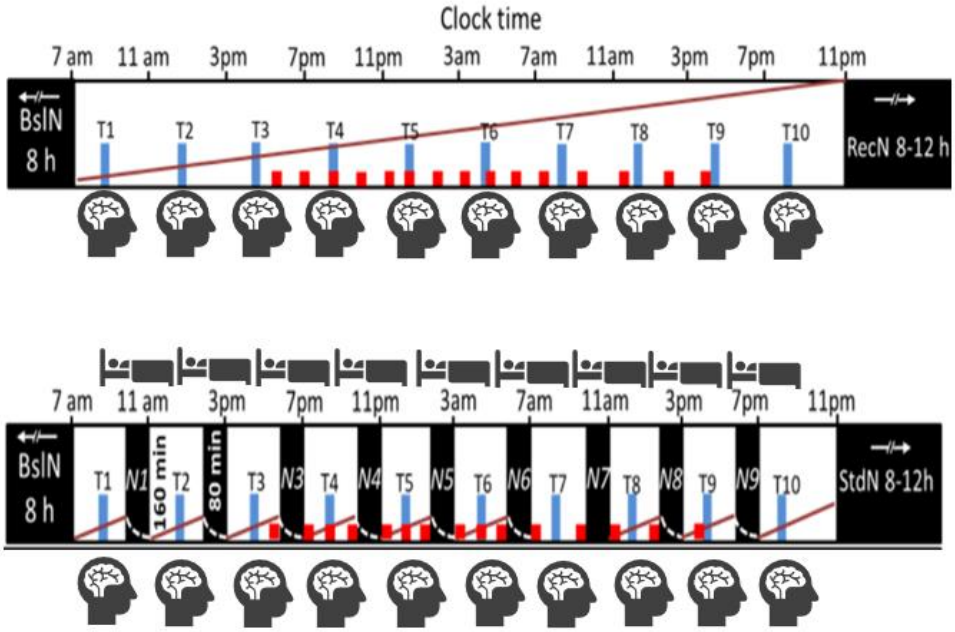


Figure 20. The study paradigm. Participants underwent a 70h-long laboratory session. After a baseline night (BslN), the participants were randomly assigned to either 40-h total sleep deprivation or multi-nap experimental conditions followed by the recovery night (StdN). Cognitive assessments were run every 4 hours (blue bars – T1-T10). Red bars indicate melatonin sampling. The burgundy lines show sleep pressure, expected to increase steadily in the SD protocol (upper panel) and maintain lower levels in the MN condition (lower panel). Nine naps were scheduled every 160 minutes and each nap was 80 minutes long (N1-N9).

5.7.1.A. Sleep deprivation protocol

During the protocol, the participants were asked to stay awake for 40 consecutive hours. If requested, in case of failing to cope with the extended wakefulness, participants were allowed to take a 4h long nap[64]. The rationale for adopting this procedure was to investigate the effect of gradually increasing sleep pressure and circadian rhythmicity on cognition, well-being and brain activity. The

[63] Analysis of the Reward Prediction task are not included in the scope of the PhD thesis.
[64] None of the participant has requested such a nap.

paradigm allowed us to investigate how the APOE-ε genotype modulates the effects of increasing sleep loss and time of the day on the main outcome measures.

5.7.1.B. Multinap Study Protocol

In the Multinap protocol, repeated 80 minutes long sleep opportunities (naps) separated by 160 minutes of out-of-bed wakefulness were scheduled throughout 40h. Repeated naps are used to keep the homeostatic sleep pressure at a relatively constant and low level compared to the sleep deprivation protocol. It also provides a unique experimental setting to investigate the contribution of the circadian system to sleep, waking cognition and brain activity (e.g., Graw et al., 2004, Cajochen et al., 2001).

5.8.2. Cognitive assessment – sleep lab session

Each cognitive assessment during the laboratory session started with the Karolinska Drowsiness Task (KDT; Kaida et al., 2006) and was followed by a Psychomotor Vigilance Task (PVT; Dinges & Powell, 1985), n-back task (1 and 2 back conditions, Kirchner, 1958), Pursuit Tracking task[65] (Lo et al., 2012, Frith, 1973 and Maquet et al., 2003)[66] and 10-minutes-long break (Figure 21). The second part of the assessment was randomized for each participant to avoid the order effect and included Sea Hero Quest (SHQ) (Coutrot et al., 2018), Episodic memory task and Semantic Priming task (Renoult et al., 2012)[21]. Each cognitive session was finalized with KDT and Static Posturography task[21]. The training session included all the cognitive tasks and was run before the baseline night to make sure that all the instructions are clear and the participant feels comfortable with all of the tasks. An overview of analysed outcome measures can be found in Table 10.

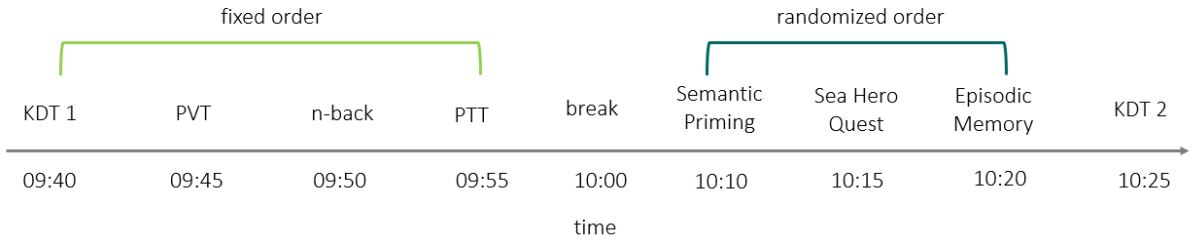


Figure 21. Cognitive test battery (COGT). The cognitive tasks used during the sleep lab protocol with the corresponding time scale indicating the duration of each task. The first block of the tasks was administered always in a fixed order, while the tasks following the break were randomized for each participant. Karolinska Drowsiness Test 2 was given always at the end of the session.

The tasks used in the first part of the cognitive battery are sensitive to increasing sleep pressure and are commonly used in protocols challenging sleep and wake homeostasis (e.g., Lo et al., 2012, Blatter et al., 2006, Reichert et al., 2017). On the other hand, Sea Hero Quest assessing spatial

[65] The Pursuit Tracking task is not included into the scope of presented PhD thesis as it is the main focus of another PhD project.
[66] The Posturography task is not included into the scope of presented PhD thesis as it is the main focus of another PhD project.

navigational skills and the Episodic Memory task measuring object-location-associative-memory performance are novel additions and were the main cognitive outcomes of the sleep lab session.

5.8.2.A. Karolinska drowsiness Test

The participants were instructed to look at the grey dot displayed in the middle of the black screen and refrain from blinking extensively for 2 minutes.

5.8.2.B. Psychomotor Vigilance task

The Psychomotor Vigilance Task (adapted from Dinges & Powell, 1985) is a well-established simple reaction time task assessing sustained attention. During the 5 minutes-long task, the participants were asked to focus their gaze on the white fixation cross displayed in the middle of the black screen and to keep an index finger of a dominant hand on the response key (spacebar). Once the fixation cross was replaced by a green dot (the stimulus appeared at random intervals between 2s to 10s), the participant was instructed to press the spacebar as fast as they can. After pressing the spacebar the reaction time (RT) was displayed in the middle of the screen for 1000ms. The RT $\geq$ 500ms was classified as a lapse of attention, false starts were identified when RT $\leq$ 100ms, hence a PVT response was regarded as valid if RT $\geq$ 100 ms (Basner & Dinges, 2011). Variables of interest were based on previous studies (Basner & Dinges, 2011, Thomann et al., 2014). The task was created in the E-Prime® 3 Psychological Software.

5.8.2.C. n-back task

Working memory performance was assessed using the n-back task consisting of one and two back conditions. The participants were asked to attend the continuous stream of 75 white letters displayed one at a time for 1.5ms in the middle of the black screen (inter-stimulus interval (ISI) of 500ms). In the *one-back* condition, the participant was instructed to press a response key (spacebar) when the letter presented on the screen was the same as one letter before, e.g., in the following string "L O R R " the second, underlined "R" is a target because is the same as the preceding letter. In the *two-back* condition, the participant needed to press the spacebar when the letter was the same as the letter displayed two before, e.g., in the string "M A M A" the underlined letter "M" and underlined letter "A" are the targets. No feedback was provided in either condition. A new set of stimuli was prepared for each cognitive session (t1-t10) using the stimuli sequence provided by Dr Christina Schmidt. The task was created using the E-Prime® 3 Psychological Software.

5.8.2.D. The measures of spatial navigation - Sea Hero Quest

Sea Hero Quest (SHQ) is a cognitive task aiming to provide the world's first point of reference for human spatial navigation (Coutrot et al., 2018). The task consists of 79 levels of different difficulties; the trials are getting harder along with changing landscapes (e.g., Arctic Rivers, Golden Shores, Mystic Marshes, Kand Reef). There are two different types of levels, i.e., egocentric *path integration* and allocentric *wayfinding*. In the path integration levels, the participants navigated a boat along a river with banks to find a flare gun. Once the flare was found, the player needed to shoot it to the starting point choosing one of three options indicating different directions. The player could receive up to three stars if the flare was shoot in the correct starting direction (Figure 22A). In the wayfinding levels, the participants needed to memorize a map including numbered checkpoints (Figure 22B). After encoding the map, the participants were asked to find the buoys in numerical order (Figure 22C).

Both tasks required navigating a boat via tapping the edges of the iPad to move left or right, swiping down to stop the boat and swiping up to accelerate. There was no time limit to complete the task. The participants completed the practice sessions including both types of levels to familiarize themselves with the game and the sensitivity of an iPad's screen before the baseline night. Importantly, the performance of each participant was normalized using practice levels 1 and 2 which allowed us to account for the technology proficiency of each participant.

The levels were matched according to the degree of difficulty and divided into *easy* and *difficult* ones based on performance during a pilot study on a cohort of eight young adults (see Appendix 8 - Sea Hero Quest pilot). For each COGT assessment (t1-t10), each participant performed randomly assigned one easy and one difficult flare level and one easy and one difficult wayfinding level.

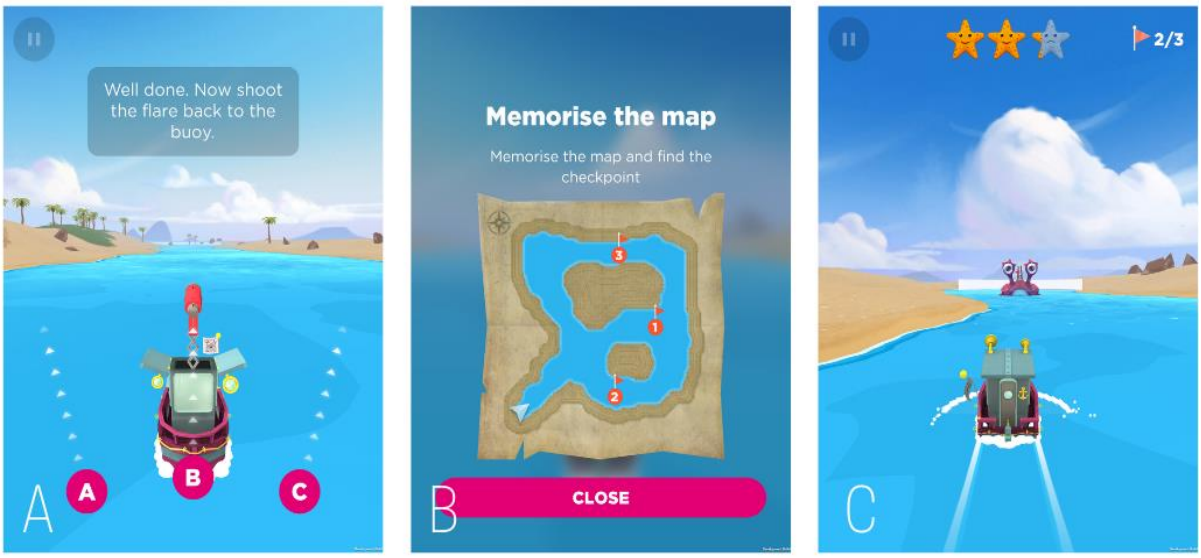


Figure 22. The Sea Hero Quest (SHQ) task. In the egocentric task, the participants were asked to explore the environment in search of a flare gun and then shoot it toward the starting point (A) In the allocentric task, the participants viewed the map and were asked to encode the checkpoints represented as buoys (B) and then navigate the boat to find them in ascending order (C) The images are the screenshot taken while performing the SHQ task (Coutrot et al., 2018).

5.8.2.E. Episodic memory task

Episodic long-term memory task (Tu et al., 2014) assessed object-location-associative-memory performance. The episodic memory assessment is currently a golden standard cognitive examination used in the diagnosis of AD, however, a growing body of evidence demonstrates that spatial navigation has the potential to detect the early stages of pre-symptomatic AD (Coughlan et al., 2018). Accordingly, in the proposed project, episodic memory was measured along with the spatial navigation task (SHQ) to investigate which task (if any) will show significant differences between ε4 allele carriers and controls (ε3/ε3).

The task consisted of *an encoding session* where the participant was asked to memorize 15 targets consisting of everyday objects, animals, food and body parts and their location on the screen (Figure 23). The interstimulus interval (ISI) was set to 500ms, whereas the stimulus presentation time to 1500ms. Right after the encoding phase, the target stimuli were intermixed with 15 distractors and the participants were asked to perform an *old-new-recognition-task* followed by a screen-location-recall-judgment, i.e., source-memory test (self-paced response). Stimuli sets were randomized for each testing session to reduce practice effects. The task was run via the NeurOn® Neuropsychology Online platform.

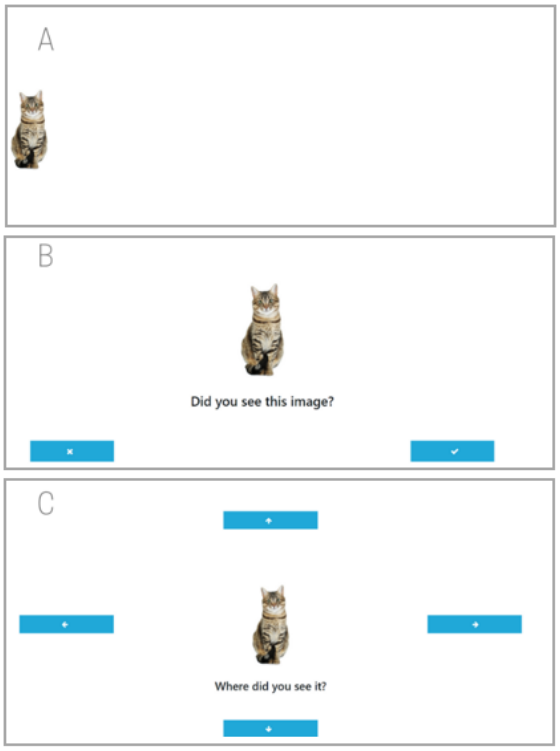


Figure 23. The Episodic memory task. The participants were asked to memorize 15 images and their location on the screen (top, bottom, left or right) (A) then the stimuli presented during the encoding session (A) were mixed with 15 new images and the participants performed the old/new recognition task (B) if the stimulus was indicated as seen, the participant needed to point where it was displayed (top, bottom, left or right), i.e., source memory task (C).

5.8.2.F. Outcome measures – cognitive tasks – sleep lab session

	Task [score range]	Assessed Cognitive function	Outcome measures
Laboratory sessions – Cognitive Assessment	Psychomotor Vigilance Task (PVT; Dinges & Powell, 1985) [0-infinity]	sustained-attention, reaction-time	- mean RT lapses included [ms] - mean RT lapses excluded [ms] - fastest 10% RT [ms] - slowest 10% RT lapses excluded [ms] - number of lapses laps = RT > 500 ms
	n-back (Kirchner, 1958) [accuracy one-back: 0-75] [accuracy two-back: 0-75]	working memory, working memory capacity	- accuracy one-back - accuracy two-back - RT one-back [ms] - RT two-back [ms]
	Episodic memory task (Tu et al., 2014) [0-15]	episodic long-term memory object-location-associative-memory performance	- time needed to complete the task [sec] old/new recognition task: - HITS RT and [%] - correct rejections RT and [%] source memory task:

			- HITS – RT and [%]
	Sea Hero Quest (SHQ; Coutrot et al., 2018) [egocentric navigation: 0-3]	spatial navigation ▪ allocentric ▪ egocentric	allocentric wayfinding: - trajectory distance - time taken to complete each level [sec] egocentric path integration: - number of gained starts - time taken to complete each level [sec]
Optional tasks	Raven Progressive Matrices (Court & Raven, 1998) [0-60]	abstract reasoning, fluid intelligence, verbal short-term memory	- number of correct responses
	Digit Span Memory Task (Wechsler, 1997) [forward score: 0-8] [backwards score: 0-8]	working memory capacity	- max digits forward score - max digits backward score - total raw score

Table 10. An overview of the cognitive tasks used during the laboratory session. Please, note that only the tasks relevant to the presented PhD project were listed. Abbreviations: *sec* – seconds, *RT* – reaction times, *ACC* - accuracy

5.8.3. Mental effort scale

Mental effort was assessed using a Visual Analogue Scale (VAS), where the participant was asked to indicate how much mental effort was required to complete each task and how cognitively demanding was the whole session. The length of the VAS line was 10 cm and the distance from the beginning of the line to the point indicated by the participant (degree of mental effort/cognitive demand) was measured in mm using a ruler.

5.8.4. Lab forms

Further, three forms were used throughout the protocols (see Appendix 9 - Lab forms), where Lab Form 1 was administered every 4 hours and measured depression, anxiety, apathy, confusion and potential sources of distractors. Lab Form 2 followed each sleep episode and consisted of selected items from the KSD followed by items from MADRE. Lab form 3 included KSS, VAS measuring satiety and food cravings, 'enthusiastic' and 'irritable' components from the Positive and Negative Affect Schedule and

VAS measuring mood, tension and physical comfort[67]. The temperature was recorded hourly along with Lab form 3, while the blood pressure was monitored after each sleep episode.

5.8.5. Electrophysiological and polysomnography data collection

The polysomnographic data were recorded using 32 electroencephalography (EEG) gold-headed electrodes mounted according to the International 10-20 system using the Embla® N7000 recording system (see the montage - Figure 24). Two EOG electrodes were fixed, one for a vertical EOG (1 cm above and 1 cm lateral of the right outer canthus) and one for a horizontal EOG (1 cm below and 1 cm lateral of the left outer canthus). Additionally, bipolar EMG electrodes were placed on the chin, and two ECG electrodes were positioned in the right and left infraclavicular fossa. For the baseline night, the PSG included also a snoring sensor, nasal cannula, thoracic belt measuring breathing effort, pulse oximeter assessing blood oxygenation level, and two leg electrodes placed 2 to 3 cm apart along the anterior tibialis on the dominant leg.

The EEG signal was referenced online to the Pz electrode (i.e., common reference) and offline re-referenced to the contralateral mastoids. Fz was used as a ground electrode. The online sampling rate was 500 Hz. To filter out line power noise, the Notch filter at 50Hz was used. The aimed impedance was <5 kΩ. The exemplary hypnograms can be seen in Figure 25.

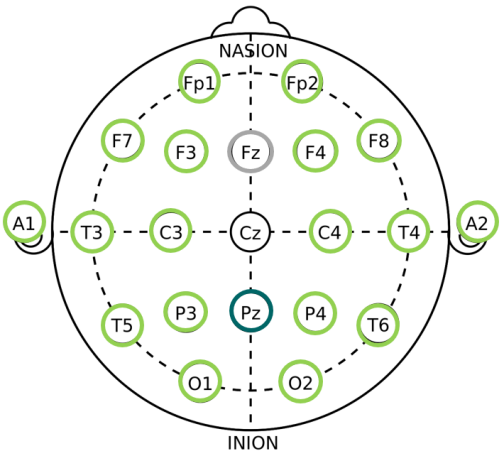


Figure 24. The EEG montage. The set-up included the following scalp electrodes (green) along with Fpz1 and Fpz2 (not displayed). Pz served as the common reference electrode (indigo), whereas Fz was the ground electrode (grey).

[67] For the scope of the thesis, only KSS variable from Lab forms 3 and KSD from Lab form 2 was used. However, during the data collection, all the answers were carefully checked to monitor the well-being of the participants.

5.8.5.A. Sleep staging

Sleep data were scored in line with the standard criteria provided by the American Academy of Sleep Medicine Scoring Manual v. 2.6 (Berry et al., 2020). Sleep episodes were sleep-staged using the *Sleep* python toolbox (Combrisson et al., 2019, Figure 25). Before sleep staging, low and high bandpass frequency filters were applied using the Butterworth method (see Table 11). All EEG electrodes were re-referenced to the contralateral mastoids. For sleep scoring, the following derivations were used F4-M1, C4-M1, O2-M1, EOG-R, EOG-L, and EMGs. Only EEG, EMG and EOG data were analysed for the presented thesis.

Signal	Low-Frequency Filter	High-Frequency Filter
EEG	0.3Hz	35Hz
EOG	0.3Hz	35Hz
EMG	10Hz	100Hz
ECG	0.3Hz	70Hz

Table 11. The following filters were applied before sleep staging. The ranges of the filters are based on the American Academy of Sleep Medicine (AASM) v.2.6 manual (Berry et al., 2020).

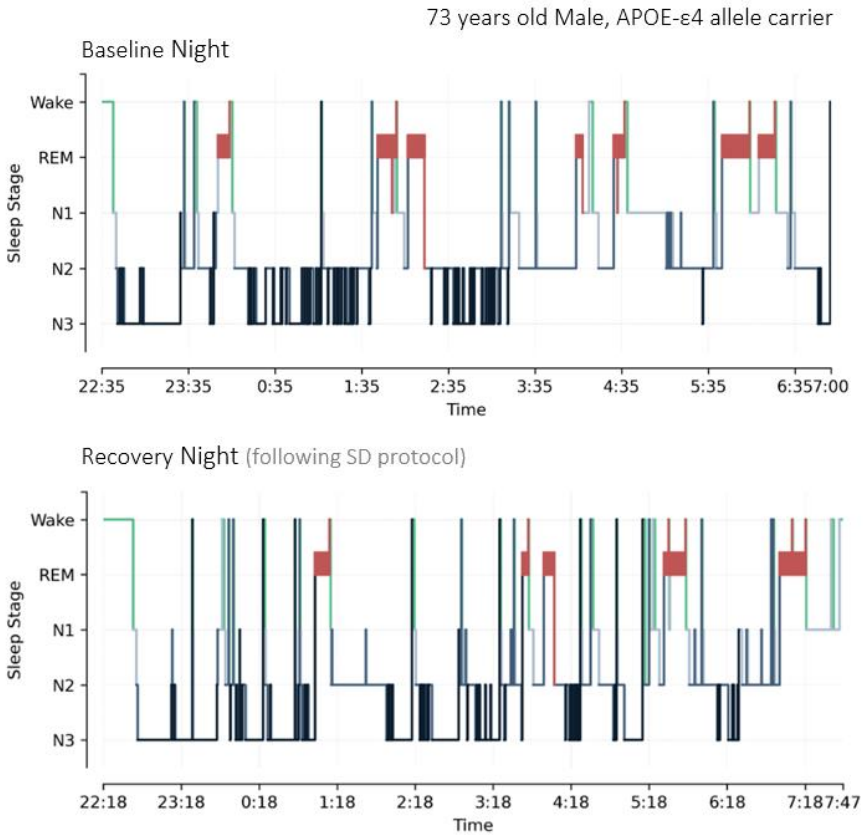


Figure 25. Exemplary hypnograms from the Baseline Night (upper hypnogram) and the recovery night (lower hypnogram).

5.8.5.B. Sleep analysis

In the presented PhD project, analyses of sleep focused on the measures of sleep architecture, sleep fragmentation and sleep continuity (Table 12). Both Baseline and Recovery nights were analysed as the whole night as well as 1st and 2nd part of the night to investigate homeostatic changes in studied sleep parameters.

	Studied sleep measures [unit]	Parameters of interest
General sleep parameters	time in bed (TIB) [min]	total time in bed in minutes, i.e., time from lights off to lights on
	total sleep time (TST) [min]	time asleep in minutes, i.e., a sum of N1, N2, N3 and REM sleep stages
	sleep period time (SPT) [min]	the time period between sleep onset time and final awakening
	sleep efficiency (SE) [%]	SE = Total Sleep Time / Total Time in Bed * 100
	wake after sleep onset (WASO) [min]	time spent awake in minutes following sleep onset
	Sleep onset latency [min]	the time until the first sleep stage (i.e. sleep onset) from lights out
	Latency to persistent sleep [min]	the time until the first five minutes of continued sleep (i.e., uninterrupted by wake episodes)
	latency to N1 [min]	the time from lights out to N1 sleep
	latency to N2 [min]	the time from lights out to N2 sleep
	latency to N3 [min]	the time from lights out to N3 sleep
	latency to REM [min]	the time from lights out to REM sleep
Sleep structure	wake duration [min]	total time spent awake in minutes
	N1 duration [min]	total time spent in N1 sleep in minutes
	N2 duration [min]	total time spent in N2 sleep in minutes

	N3 duration [min]	total time spent in N3 sleep in minutes
	REM duration [min]	total time spent in REM sleep in minutes
	N1, % of TST [%]	percentage of TST spent in N1
	N2, % of TST [%]	percentage of TST spent in N2
	N3, % of TST [%]	percentage of TST spent in N3
	REM, % of TST [%]	percentage of TST spent in REM
	Artefacts [min]	indicated unscorable epochs in mins
Sleep continuity	number of awakenings [count]	total number of awakenings, i.e., epochs scored as Wake
	awakenings from N1 [ratio]	the ratio of the total number of awakenings from N1 sleep normalized to the length of N1
	awakenings from N2 [ratio]	the ratio of the total number of awakenings from N2 sleep normalized to the length of N2
	awakenings from N3 [ratio]	the ratio of the total number of awakenings from N3 sleep normalized to the length of N3
	awakenings from REM [ratio]	the ratio of the total number of awakenings from REM sleep normalized to the length of REM
	sleep stability [ratio]	total number of sleep stage changes normalized to TST
	fast sleep stage changes [ratio]	number of 90 seconds intervals including three different sleep stages normalized to TST
	deep sleep stage changes [ratio]	number of sleep stage changes to slower brain activity level (i.e. deeper sleep), normalized to TST l i.e., the transition from N1 to N2, from N2 to N3, from REM to N1
	shallow sleep stage changes [ratio]	number of sleep stage changes to higher brain activity level (i.e. shallower sleep) normalized to TST l i.e., the transition from N1 to N2, from N2 to N3, from N1 to REM
	big deep sleep stage changes [ratio]	number of sleep stage changes normalized to TST, when the sleep deepened by more than one sleep stage, i.e., N1 to N3
	big shallow sleep stage changes [ratio]	number of sleep stage changes normalized to TST, when the sleep shallowed by more than one sleep stage, i.e., N3 to N1

Sleep fragmentation	sleep fragmentation [ratio]	number of awakenings normalized to the length of sleep duration
	N1 entries [count]	Number of entries into N1
	N1 fragmentation [ratio]	N1 fragmentation=Number of entries into N1 normalized to the length of N1.
	N2 entries [count]	number of entries into N2
	N2 fragmentation [ratio]	N2 fragmentation=Number of entries into N2 normalized to the length of N2.
	N3 entries [count]	Number of entries into N
	N3 fragmentation [count]	N3 fragmentation=Number of entries into N3 normalized to the length of N3.
	REM entries [count]	Number of entries into REM
	REM fragmentation [ratio]	REM fragmentation=Number of entries into REM normalized to the length of REM.

Table 12. Sleep macroarchitecture outcomes of interest.

5.8.6. Melatonin analysis

To investigate the value of melatonin, saliva samples were collected using Salivette® (Sarstedt, Salivette® Cortisol, Art. No. 51.1534.500). The participants were asked to chew the swab for 60 seconds during each saliva sample collection. The sampling was scheduled on the 9th, 11th, 12th, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 27th, 29th, 31st hour of the SD protocol (19 samples) and 9th, 10th, 12th, 13th, 14th, 16th, 17th, 18th, 20th, 21st, 22nd, 24th, 25th, 26th, 28th, 30th of MN protocol (16 samples) (Figure 26). The saliva collection was scheduled before the snacks to avoid contamination. Samples were further processed by the team of Dr Jonathan Tang, at the Norwich Medical School using Melatonin direct Saliva ELISA (Melatonin direct Saliva ELISA, IBL International, Version 2015-0).

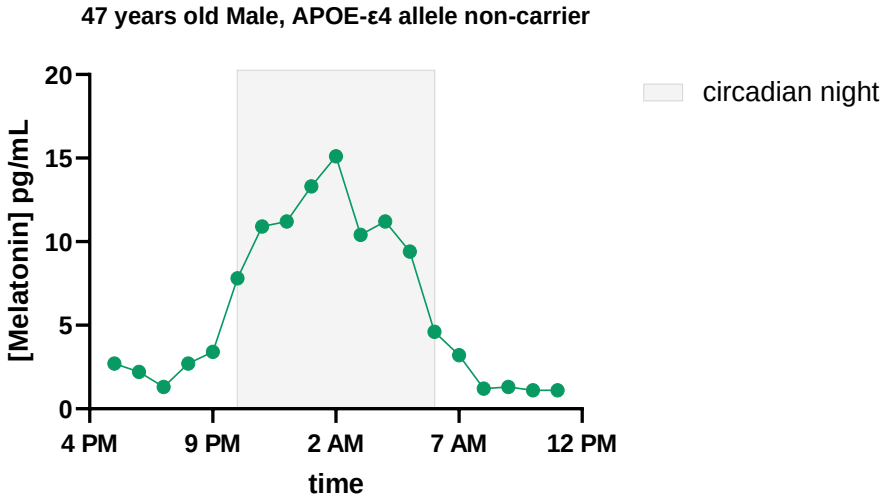


Figure 26. Exemplary melatonin profile of a single participant. The rectangle indicates the habitual sleep and wake timing of the participant (i.e., bedtime:10PM, wake-up time: 6 AM).

5.8.7. Sample size consideration and statistical analysis

Based on a pilot study comparing sleep across the APOE-ε3/3 and APOE-ε 3/4 genotypes in healthy adults (Drogos et al., 2016) the total sample size required to obtain a significant (p<0.05) genotype difference in sleep efficiency at a statistical power of 80% is 52 for actigraphy and 36 for sleep laboratory sessions (polysomnography). The planned sample size was in line with similar experimental protocols looking at genotype effects on sleep and cognition (Lo et al., 2012).

For statistical analysis, the first step was to compare APOE-ε4+ vs. APOE-ε4- using the independent samples t-test or Mann-Whitney U test depending on normality. Then ANCOVA was run to make a comparison between the genotypes while controlling for the effect of known confounders like age, biological sex and APOE-ε4 carriership. To evaluate the associations between variables of interest, correlational analysis (Spearman's rho correlational coefficient) was conducted. For comparisons between Spearman's rho, Fisher 1925 was used. For the outcomes that are measured at multiple time points, e.g., ten cognitive assessments performed during the sleep laboratory sessions, mixed models were used to account for the repeated measures of the data; fixed effects included variables like age, biological sex, genotype, time of day and protocol, whereas participant was a random effect.

To account for the multiple comparisons problem, the significance of differences was compared with and without adjustment for multiple testing using Benjamini-Hochberg correction (Benjamini&Hochberg, 1995). The level of significance (p-value) of 0.05 was used to reject H0.

In the laboratory session's section, cognitive outcomes are expressed as a ratio to the first cognitive assessment following the Baseline Night (t1), i.e., each assessment (t2-t10) was divided by t1 performance. To acknowledge inter-individual differences, each data point of a given participant was divided by hers/his baseline task performance (t1).

5.8.8. Distribution diagnostics and statistical transformations

Distributions and skewness of the raw data and residuals in case of multivariate analyses were assessed by plotting histograms, box plots and Q-Q plots. The Shapiro-Wilk test was run to assess normality. In the case of right-skewed data, log transformation was applied. Left-skewed data were normalized using power transformation whereas outcome measures expressed as ratios (e.g. percentages) were transformed by logit transformation.

5.8.9. GitHub repository

For full transparency, all the scripts used for data manipulations and analysis can be found in the GitHub repository of Adriana Michalak (https://github.com/MissAdrianaMichalakInJourney).

CHAPTER VI – Results

6.1. Participants

Data collection has taken place from January 2019 to July 2021. One-hundred-sixty-six English-speaking participants who met entry eligibility criteria (see Methods-*Recruitment*) were invited to participate in the screening session (M=49, F=117, age(*M*±*SD*): 65±9) of which 58 completed the actigraphy session (M=22, F=36, age(*M*±*SD*): 65±9) and 38 took part in the laboratory session (M=17, F=20, age(*M*±*SD*): 65±9). All participants involved in the study identified themselves as *White British* except one who selected the option: *White Other*. Each participant identified themselves as either male or female (option *other* was included). Further, three sleep laboratory sessions were terminated, one at the request of the participant and two others due to medical concerns.

6.2. APOE genotype groups

For the analysis of the screening data APOE-ε4 allele carriers, i.e., participants at the increased genetic risk of AD were defined as individuals with either ε3/ε4 (n=44) or ε4/ε4 (n=7) genotypes, while non-carriers, i.e., controls who are individuals that do not represent an elevated risk of AD being ε2/ε3 (n=13) and ε3/ε3 (n=97) carriers. Participants with genotype ε2/ε4 (n=5) were excluded from the analysis because of a lack of consensus in the literature if those participants should be considered at increased, lower or neutral risk for AD (e.g., Insel et al., 2020, Goldberg et al., 2020). For the field and sleep laboratory sessions controls included only individuals with ε3/ε3 genotype and the ε4 carriers were defined as individuals with ε3/ε4 or ε4/ε4 genotypes.

6.3. Results section arrangement

The results section is divided into three parts, i.e., screening session, field session (actigraphy session) and laboratory session. Each section starts with a table featuring basic demographics, psychological, sleep, sleepiness, chronotype and cognitive characteristics of APOE-ε4 allele carriers and non-carriers and is followed by analyses aiming to address research questions stated in the *Objectives and Research Questions* chapter. To avoid exploratory analysis elevating the risk of type I error, all the analyses were strictly driven by research questions. To further compensate for a large amount of conducted statistical tests, each *p*-value is accompanied by an adequate effect size estimate. The effect sizes interpretation, i.e., none, small, medium and large is based on a paper by Maher et al. (2013). Significant *p* values, i.e., $p \leq 0.05$ were highlighted in dark grey, while trends towards significance are emphasised in light grey. The adjusted *p*-values were calculated using the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1994) wherever applicable (the procedure was not run for multivariate analysis as the effects were not independent of each other).

6.4. Screening session

6.4.1. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – APOE-ε4+ vs. ε4- allele carriers

The two genotype groups were similar in most studied features in the screening phase. The only significant differences between ε4 allele carriers and non-carriers obtained by performing independent samples t-test or Mann-Whitney U test were in years of education, anxiety and general cognitive status as measured by GAD-7 and m-ACE, respectively (Table 13). Based on these contrasts, ε4 allele carriers were less anxious, spent more years in education and had a higher global cognitive score as measured by m-ACE. There was also a non-significant trend showing that at-risk participants tend to overperform the controls (ε4 allele non-carriers) in visual-spatial attention and visual memory tasks (TM-Part A - errors and time to complete, ROCF - Immediate and Delayed recalls). The at-risk group was also slightly younger. However, none of the mentioned effects survived adjustment for multiple comparisons.

All the effect sizes (Table 13) ranged between negligible to small with years of education (*d*=0.35) and psychomotor speed assessed by TMT Part A (time to complete) (*d*=0.33) indicating the largest effect sizes. Spatial navigation outcome measures reached insignificant Cohen's *d* values.

Outcome measures [range]	*APOE-ε4+ (N=51) (M (SD)*	*APOE-ε4- (N=110) (M (SD)*	*Total (N=161) (M (SD)*	*p*	*p-adj*	*Effect size*
Age [years]	63.18 (7.84)	65.66 (9.98)	64.88 (9.41)	0.09 [b]	0.33	0.16
Sex [female N[%]]	36 (70.59%)	77 (70%)	113 (70.19%)	0.94[c]	1.00	0.01
Years of education	16.92 (4.37)	15.63 (3.43)	16.03 (3.78)	0.05 [a]	0.48	0.35
Self-reported hours of sleep	6.82 (0.90)	6.71 (1.09)	6.74 (1.03)	0.54 [a]	0.87	0.11
PSQI [sleep quality] [0-21]	5.78 (3.28)	6.06 (3.30)	5.97 (3.29)	0.46 [b]	0.83	0.07
midpoint of sleep	04:04 AM	04:08 AM	04:07 AM	-	-	-
ESS [sleepiness] [0-24]	4.53 (2.44)	5.40 (3.59)	5.12 (3.28)	0.19 [b]	0.50	0.13
ISI [insomnia] [0-28]	5.47 (5.03)	6.69 (5.61)	6.30 (5.45)	0.21 [b]	0.51	0.12
MEQ [chronotype] [16-86]	60.75 (7.12)	61.08 (8.32)	60.97 (7.93)	0.59 [b]	0.78	0.05
PHQ-9 [depression] [0-27]	1.82 (2.46)	2.44 (2.81)	2.24 (2.72)	0.12 [b]	0.39	0.15
GAD-7 [anxiety] [0-21]	1.33 (2.45)	2.61 (3.97)	2.20 (3.60)	0.02 [b]	0.58	0.21
CCI total [subjective cognitive decline] [20-100]	31.06 (10.58)	31.52 (10.20)	31.37 (10.29)	0.65 [b]	0.82	0.05
CCI memory [12-60]	20.02 (6.44)	20.93 (7.19)	20.64 (6.95)	0.48 [b]	0.82	0.07
CCI executive functions [5-25]	6.04 (2.76)	5.64 (2.34)	5.76 (2.48)	0.22 [b]	0.49	0.11
CCI language [3-15]	4.16 (1.84)	4.13 (1.75)	4.14 (1.77)	0.92 [b]	0.92	0.01
m-ACE [global cognitive status] [0-30]	28.80 (1.47)	28.19 (1.97)	28.39 (1.84)	0.04 [b]	0.58	0.19

SDMT [speed of processing] [0-110]	49.48(7.85)	47.94 (8.55)	48.407 (8.25)	0.54 [b]	0.82	0.06
TMT Part A (sec) [visuospatial attention]	27.51 (8.24)	30.96 (11.45)	29.86 (10.63)	0.06 [a]	0.44	0.33
TMT Part A errors [visuospatial attention] [0-22]	0.12 (0.33)	0.34 (0.71)	0.27 (0.62)	0.06 [b]	0.35	0.13
TMT Part B (sec) [visuospatial attention]	60.67 (22.35)	67.06 (28.11)	64.98 (26.48)	0.16 [a]	0.46	0.24
TMT Part B errors [visuospatial attention] [0-24]	0.32 (0.65)	0.35 (0.85)	0.34 (0.79)	0.89 [b]	0.92	0.01
HVLT - Total Recall [verbal memory] [0-36]	26.41 (4.34)	26.09 (4.85)	26.19 (4.68)	0.83 [b]	0.93	0.02
HVLT - Delayed Recall [verbal memory] [0-12]	9.47 (1.95)	9.21 (2.07)	9.30 (2.03)	0.56 [b]	0.81	0.06
HVLT – Recognition [verbal memory] [0-12]	10.94 (1.08)	10.98 (1.29)	10.95 (1.23)	0.38 [b]	0.79	0.08
ROCF – Copy [visuospatial abilities] [0-36]	33.38 (3.14)	33.84 (2.21)	33.69 (2.54)	0.57 [b]	0.79	0.06
ROCF - Immediate Recall [visual memory] [0-36]	19.53 (6.66)	17.21 (7.33)	17.96 (7.18)	0.06 [b]	0.29	0.19
ROCF - Delayed Recall [visual memory] [0-36]	19.40 (6.77)	17.21 (6.51)	17.92 (6.66)	0.07 [b]	0.29	0.18
ROCF – Recognition [visual memory] [0-24]	19.84 (2.25)	19.98 (2.30)	19.94 (2.28)	0.87 [b]	0.93	0.02
VST - Egocentric Navigation [0-7]	2.68 (1.62)	2.76 (1.55)	2.73 (1.57)	0.67 [b]	0.81	0.04
VST - Allocentric Navigation	15.74 (8.45)	14.69 (7.34)	15.05 (7.72)	0.44 [a]	0.85	0.14
VST - Heading Direction [spatial navigation] [0-7]	5.44 (1.47)	5.54 (1.33)	5.51 (1.38)	0.81 [b]	0.94	0.02

Table 13. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics based on selected screening session's outcome measures. Statistical tests and measures of effect sizes: a – independent sample t-test & Cohen's d), b – Mann-Whitney U Test & Rank-Biserial Correlation, c – Chi^2 & Cramer's V; Abbreviations: *CCI* - Cognitive Change Index; *PHQ-9* - Patient Health Questionnaire 9; *GAD-7* - General Anxiety Disorder 7; *PSQI* - Pittsburgh Sleep Quality Index; *ISI* - Insomnia Severity Index; *ESS* - Epworth Sleepiness Scale; *MEQ* – Morningness Eveningness Questionnaire; *m-ACE* - mini Addenbrooke's Cognitive Examination; *SDMT* - Symbol Digits Modalities Test; *TM* - Trail Making Test; *HVLT* - Hopkins Verbal Learning Test; *ROCF* - Rey Osterrieth Complex Figure; *VST* – Virtual Supermarket Task; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj)

6.4.2. Does APOE-ε4 allele carriership affect self-reported sleep, chronotype, and cognition independently of potential confounding factors such as age, and biological sex?

ANCOVA including APOE-ε4 allele carriership (binary variable, i.e., ε4 allele carriers vs non-carriers) and sex as *fixed factors* and age as a *covariate* was run for self-reported hours of sleep, PSQI total score, ISI, MEQ and cognitive assessment outcomes. While age and sex had an independent significant effect on multiple outcome measures, this was not the case for the APOE genotype which showed only a trend toward significance for m-ACE (p=0.09), where ε4 carriers overperformed the controls (ε4 allele non-carriers) (ε4+ ($M \pm SD$): 28.80 ± 1.47; ε4- ($M \pm SD$): 28.19 ± 1.97) (Table 14). A significant effect of ε4 status was found for none of the spatial navigation outcome measures. Yet, age was a significant predictor for egocentric response (p=0.01), allocentric navigation ($p \leq 0.001$) and heading direction (p=0.003), while biological sex yielded significance for allocentric navigation (p=0.001). Overall, women performed worse in allocentric navigation compared to men (women ($M \pm SD$): 15.91 ± 7.73; men ($M \pm SD$): 12.76 ± 7.11)). The analysis was not run for ESS, TMT Part A and B

errors as well as Hopkins Recognition as their residuals were strongly skewed and failed to be normalized using data transformations.

For predictor age, the effect sizes listed in Table 14 ranged from small to large with the speed of processing (SDMT, η^2=0.30), visuospatial attention (TM Part A (sec) (η^2=0.22) and TM Part B (sec) (η^2=0.18)) and allocentric navigation (η^2=0.17) having the largest effect sizes. The eta squares for predictor sex were small besides the large effect of verbal memory (HVLT – Delayed recall, η^2=0.66) and allocentric navigation (VST, η^2=0.06). Effect sizes for predictor APOE-ε4 status ranged from none to small for all dependent variables. Among spatial navigation outcome measures, only allocentric navigation reached a small effect size (η^2=0.01).

Dependent variable	*Predictors*								
	Age			Sex			APOE-ε4 status		
	F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
PSQI [sleep quality]	3.33	0.07	0.02	8.98	0.003	0.05	0.111	0.74	0.00
Self-reported hours of sleep	1.76	0.19	0.01	2.13	0.15	0.01	0.200	0.67	0.00
ISI [insomnia]	0.01	0.94	0.00	2.90	0.09	0.02	1.793	0.18	0.01
MEQ [chronotype]	0.46	0.50	0.00	3.01	0.09	0.02	0.022	0.88	0.00
CCI total [subjective cognitive decline]	6.04	0.02	0.04	0.18	0.67	0.00	0.002	0.97	0.00
CCI memory	5.54	0.02	0.03	0.18	0.67	0.00	0.23	0.63	0.00
CCI executive functions	1.52	0.22	0.01	0.00	0.97	0.00	1.22	0.27	0.01
CCI language	4.31	0.04	0.03	0.33	0.57	0.00	0.13	0.72	0.00
m-ACE [global cognitive status]	5.33	0.02	0.03	0.67	0.42	0.00	2.91	0.09	0.02
SDMT [speed of processing]	67.58	<.001	0.30	1.22	0.27	0.01	0.05	0.82	0.00
TMT Part A (sec) (log10) [visuospatial attention]	44.03	<.001	0.22	0.73	0.39	0.00	1.89	0.17	0.01
TMT Part B (sec) (log10) [visuospatial attention]	33.61	<.001	0.18	0.58	0.50	0.00	0.95	0.33	0.01
HVLT - Total Recall [verbal memory]	12.89	<.001	0.07	21.69	<.001	0.11	0.00	0.99	0.00
HVLT - Delayed Recall [verbal memory]	8.46	0.004	0.05	11.37	<.001	0.66	0.23	0.632	0.01
ROCF – Copy [visuospatial abilities]	8.87	0.003	0.05	0.413	0.52	0.00	2.05	0.15	0.01
ROCF - Immediate Recall [visual memory]	16.91	<.001	0.09	7.22	0.01	0.04	2.45	0.12	0.01
ROCF - Delayed Recall [visual memory]	13.18	<.001	0.08	6.39	0.01	0.04	2.74	0.10	0.02
ROCF – Recognition [visual memory]	6.63	0.01	0.04	0.81	0.37	0.01	0.42	0.52	0.00
VST - Egocentric Navigation (VST-E)	6.77	0.01	0.05	0.01	0.94	0.01	0.29	0.59	0.00
VST - Allocentric Navigation (VST-A)	32.4	<.001	0.17	10.62	0.001	0.06	1.93	0.17	0.01
VST - Heading Direction (VST-HD)	9.06	0.003	0.06	0.15	0.70	0.00	0.52	0.47	0.00

Table 14. ANCOVA outcomes for self-reported sleep, sleepiness, chronotype and cognitive outcome measures. Statistical test and measure of effect size: ANCOVA & eta squared

6.4.3. What is the association between spatial navigation, self-reported sleep, chronotype and cognition?

Spearman's rank correlation coefficient (Spearman's rho) was used to assess the degree of associations between self-reported sleep, chronotype, cognition and spatial navigation (VST) (Figure 27). The *p*-value threshold was kept at 0.01 to avoid taking into account very weak associations (i.e., Spearman's rho=0.01-0.19 being considered '*no or negligible relationship*'). No significant associations between either sleep or chronotype variables and Virtual Supermarket task outcome measures were found.

No associations were found between subjective measures of chronotype (MEQ) and subjective (CCI) and objective cognitive outcomes. Yet, low sleep quality assessed by PSQI (global score) and a higher level of sleepiness (ESS) were correlated with more severe subjective impairment (CCI – total score as well as memory, executive functions and language subcategories). Further, a higher rating of insomnia severity (ISI) was associated with poorer verbal memory (HVLT – Recognition) and a higher rating of subjective impairment (CCI total score and executive functions).

As summarized in Table 15, better performance in the VST head direction task (VST-HD) and VST allocentric task (VST-A) was significantly associated with lower age, better speed of processing (SDMT performance) and more efficient visual memory (ROCF - Immediate and Delayed recalls). Further, better VST-HD performance was correlated with better visuospatial abilities (ROCF - Copy), while better VST-A execution was associated with better visual memory (ROCF - Recognition) and more efficient visuospatial attention (TM-Part A (sec) and B (sec)). More efficient VST-HD performance was associated with better VST-A performance (Spearman's rho = -0.44, $p \leq 0.001$).

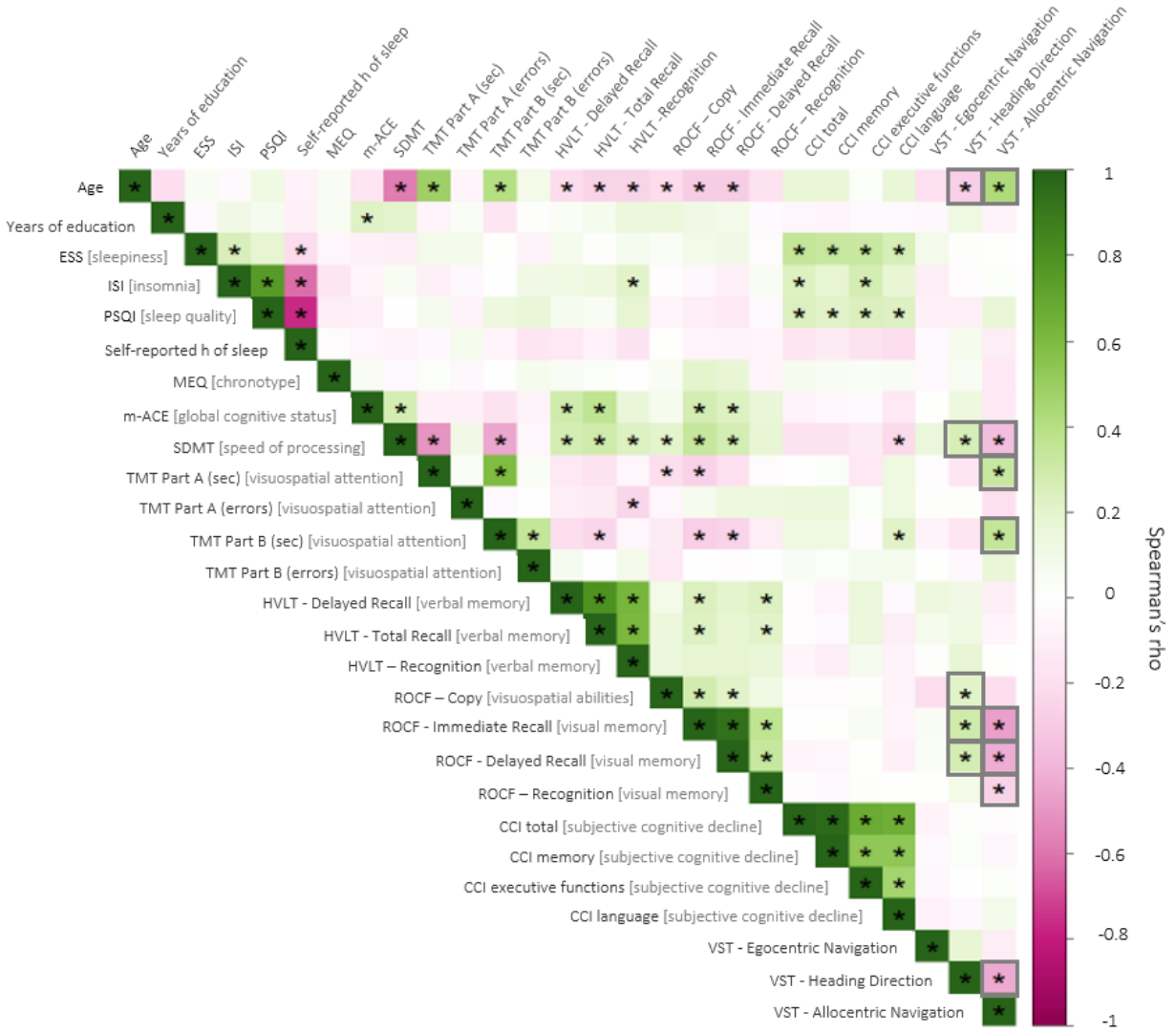


Figure 27. Adjacency matrix showing correlations between all studied cognitive and self-reported sleep, sleepiness and chronotype measures (n=161). Stars indicate significant correlations (p<0.01). The colour scale indicates the strength and direction of the association. Significant VST correlations that survived Benjamini-Hochberg's correction are highlighted in grey.

Variables	Heading Direction (VST-HD)			Allocentric Direction (VST-A)		
	Spearman's rho	*p*	*p-adjusted*	*Spearman's rho*	*p*	*p-adjusted*
Age	-0.26	<.001	0.01	0.42	<.001	<.001
SDMT [speed of processing]	0.26	0.001	0.01	-0.36	<.001	<.001
TMT Part A (sec) [visuospatial attention]	-	-	-	0.37	<.001	<.001
TMT Part B (sec) [visuospatial attention]	-	-	-	0.35	<.001	<.001
ROCF – Copy [visuospatial abilities]	0.22	0.008	0.04	-	-	-
ROCF - Immediate Recall [visual memory]	0.29	<.001	0.00	-0.47	<.001	<.001
ROCF - Delayed Recall [visual memory]	0.28	<.001	0.00	-0.42	<.001	<.001
ROCF – Recognition [visual memory]	-	-	-	-0.26	0.002	0.01

Table 15. Significant correlations for Heading direction and Allocentric outcomes of the Supermarket task (demonstrated in Figure 27). Benjamini-Hochberg procedure was applied to calculate adjusted p values (p-adj).

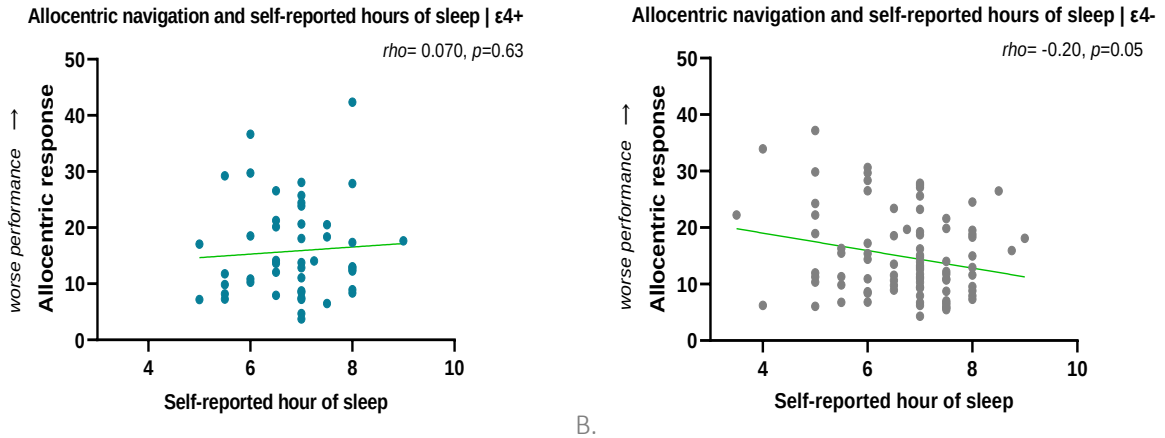
6.4.4. Does the APOE-ε4 allele carriership modulate associations between self-reported sleep, chronotype and spatial navigation?

Spearman's correlation involving self-reported sleep, chronotype and cognitive outcome measures was run independently for APOE-ε4 allele carriers and non-carriers (Table 16). The correlation coefficients were compared using Fisher's *r*-to-*Z* transformation. The only significant differences between correlations coefficients were found for associations between egocentric navigation (VST-E) and attention/visual search (TMT Part A – errors), as well as Heading Direction (VST-HD) and visual memory (ROCF - Immediate Recall). In both cases, the associations were stronger for ε4+ (VST-E x TMT Part A (errors): *rho* = -0.36; VST-HD x ROCF Immediate Recall: *rho* = 0.49) compared to ε4- (VST-E x TMT Part A (errors): *rho* = 0.14; VST-HD x ROCF Immediate Recall: *rho* = 0.19). Separate correlational plots for ε4+ and ε4- allele carriers can be found in Appendix 10 - *Associations between self-reported sleep, chronotype and cognitive measures in APOE-ε4+ and non-carriers.* There was a marginally significant positive association between self-reported hours of sleep and allocentric navigation performance (i.e., longer time asleep is associated with better allocentric performance), yet in the control group only (Table 16, Figure 28A, 28B).

Outcome measures	Egocentric Navigation (VST-E)			Heading Direction (VST-HD)			Allocentric Navigation (VST-A)		
	z	*p*	*p*-adj	*z*	*p*	*p*-adj	*z*	*p*	*p*-adj
Age [years]	0.75	0.45	1.00	-0.19	0.85	0.85	0.20	0.84	1.00
Years of education	-1.23	0.22	1.00	1.12	0.26	0.85	-0.09	0.92	1.00
Self-reported hours of sleep	-0.01	0.99	0.99	0.98	0.33	0.95	1.59	0.11	1.00
PSQI [sleep quality]	0.69	0.49	1.00	-0.79	0.43	0.86	-0.58	0.56	1.00
ESS [sleepiness]	0.42	0.67	1.00	0.52	0.60	0.87	0.45	0.65	1.00
ISI [insomnia]	0.34	0.73	1.00	-0.23	0.82	0.89	-0.14	0.89	1.00
MEQ [chronotype]	0.51	0.61	1.00	-0.86	0.39	0.85	-0.31	0.75	1.00
CCI total [subjective cognitive decline]	-0.35	0.72	1.00	0.33	0.74	0.87	0.32	0.75	1.00
CCI memory	-0.27	0.79	1.00	1.19	0.24	0.89	0.12	0.91	1.00
CCI executive functions	-0.74	0.46	1.00	-0.41	0.68	0.88	0.22	0.83	1.00
CCI language	0.59	0.56	1.00	-1.29	0.20	1.00	0.34	0.74	1.00
m-ACE [global cognitive status]	0.29	0.80	0.99	1.54	0.12	1.00	-1.41	0.16	1.00
SDMT [speed of processing]	0.41	0.69	1.00	0.40	0.69	0.85	-0.02	0.98	1.00
TMT Part A (sec) [visuospatial attention]	-0.34	0.74	1.00	-1.31	0.19	1.00	1.12	0.26	1.00
TMT Part A errors [visuospatial attention]	-2.96	0.003	0.08	-0.28	0.78	0.88	-0.04	0.97	1.00
TMT Part B (sec) [visuospatial attention]	-0.15	0.88	0.99	-0.22	0.83	0.86	-0.05	0.96	1.00
TMT Part B errors [visuospatial attention]	0.95	0.34	1.00	0.51	0.61	0.83	-1.32	0.19	1.00

HVLT - Total Recall [verbal memory]	-1.33	0.18	1.00	-0.97	0.33	0.86	0.65	0.51	1.00
HVLT - Delayed Recall [verbal memory]	-0.91	0.36	1.00	-1.32	0.19	1.00	0.94	0.34	1.00
HVLT – Recognition [verbal memory]	0.25	0.77	1.00	-0.74	0.46	0.80	1.53	0.13	1.00
ROCF – Copy [visuospatial abilities]	0.20	0.84	0.99	-0.92	0.37	0.87	0.67	0.50	1.00
ROCF - Immediate Recall [visual memory]	1.64	0.10	1.00	1.98	0.05	1.00	0.36	0.72	1.00
ROCF - Delayed Recall [visual memory]	1.51	0.13	1.00	1.19	0.23	1.00	0.57	0.57	1.00
ROCF – Recognition [visual memory]	0.14	0.89	0.96	-0.74	0.46	0.75	0.60	0.55	1.00
VST - Egocentric Navigation	-	-	-	0.63	0.53	0.81	-0.14	0.89	1.00
VST - Allocentric Navigation	-0.14	0.89	0.93	-0.76	0.45	0.84	-	-	-
VST - Heading Direction [spatial navigation]	0.63	0.53	1.25	-	-	-	-0.76	0.45	1.00

Table 16. Comparison of Spearman's rho correlation coefficients between ε4 allele carriers and non-carriers involving associations between Virtual Supermarket task outcome measures and screening variables of interest. Statistical test and measure of effect size: Fisher 1925. Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).



A. B.

Figure 28. The association between self-reported hours of sleep and allocentric response (VST-A) in ε4 allele carriers (A) and non-carriers (B). The regression lines shall not be interpreted in the context of reported Spearman rho as it is used solely for showing the direction of the relationship.

SUMMARY of FINDINGS – Screening session

While controlling for age and sex, APOE-ε4 status showed no independent main effect on self-reported sleep (PSQI, ISI), sleepiness (ESS), chronotype (MEQ) and cognition (SDMT, TMT, HVLT, ROCF) besides marginally better global cognitive status (m-ACE) in ε4 allele carriers. The spatial navigation performance assessed by the Virtual Supermarket Task was not significantly different between ε4 allele carriers and non-carriers.

Further, better allocentric navigation (VST-A) was correlated with faster speed of processing (SDMT), more efficient visuospatial attention (TM-A and B), better visual memory (ROCF) and lower age.

Similarly, better heading direction performance (VST-HD) was associated with lower age, faster speed of processing (SDMT), better visual memory and visuospatial abilities (ROCF).

The interrelationship between self-reported sleep, chronotype and spatial navigation was not shown to be modulated by APOE-ε4 allele status, except for a weak association between longer subjective sleep duration and better allocentric navigation performance (VST-A) in non-carriers.

6.5. Actigraphy session

6.5.1. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – ε4+ vs. ε4- allele carriers

In the subgroup of screened participants who also took part in the field session, the APOE-ε4 allele carriers and non-carriers were comparable considering all variables of interest except years of education, where the at-risk participants had higher educational attainment relative to controls (ε3/ε3) (ε4+(*M*±*SD*): 16.36±4.06; ε4-(*M*±*SD*): 14.57±2.65) (Table 17). Effect sizes in the actigraphy session cohort fluctuated between nonsignificant to small, with only *years spent in education* reaching medium effect size (*d*=0.57).

Outcome measures [range]	*APOE-ε4+ (N=29) (M (SD)*	*APOE-ε4- (N=30) (M (SD)*	*Total (N=59) (M (SD)*	*p*	*p-adj*	*Effect size*
Age [years]	64.45 (7.36)	65.23 (10.34)	64.85 (8.93)	0.74[a]	1.00	0.09
Sex [female N[%]]	19 (65.52%)	18 (60%)	37 (62.71%)	0.66[c]		0.06
Years of education	16.36 (4.06)	14.57 (2.65)	15.43 (3.49)	0.05[a]	1.00	0.57
Self-reported hours of sleep	7.26 (0.75)	7.34 (0.69)	7.30 (0.72)	0.68[a]	1.00	0.11
PSQI [sleep quality] [0-21]	4.07 (1.87)	3.97 (2.11)	4.02 (1.98)	0.85[b]	1.00	0.03
ESS [sleepiness] [0-24]	4.34 (2.36)	4.27 (2.85)	4.31 (2.60)	0.89[b]	0.99	0.02
ISI [insomnia] [0-28]	2.97 (2.57)	3.07 (3.47)	3.02 (3.04)	0.60[b]	1.00	0.08
MEQ [chronotype] [16-86]	59.86 (7.48)	61.67 (6.84)	60.78 (7.16)	0.23[b]	1.00	0.18
PHQ-9 [depression] [0-27]	0.83 (1.36)	0.50 (0.90)	0.66 (1.15)	0.44[b]	1.00	0.10
GAD-7 [anxiety] [0-21]	0.45 (0.83)	1.00 (1.53)	0.73 (1.26)	0.19[b]	1.00	0.17
CCI total [subjective cognitive decline] [20-100]	29.97 (8.00)	28.73 (6.85)	29.34 (7.40)	0.53[b]	1.00	0.09
CCI memory [12-60]	19.48 (4.98)	18.80 (4.77)	19.14 (4.84)	0.59[b]	1.00	0.09
CCI executive functions [5-25]	6.00 (2.55)	5.20 (1.69)	5.59 (2.17)	0.10[b]	1.00	0.24
CCI language [3-15]	3.93 (1.33)	3.83 (1.32)	3.88 (1.31)	0.66[b]	1.00	0.06
m-ACE [global cognitive status] [0-30]	28.66 (1.59)	28.60 (1.45)	28.63 (1.51)	0.77[b]	1.00	0.04
SDMT [speed of processing] [0-110]	48.76 (7.09)	48.20 (8.3)	48.48 (7.96)	0.86[b]	1.00	0.03
TMT Part A (sec) [visuospatial attention]	28.03 (5.51)	30.76 (12.42)	29.40 (9.62)	0.90[b]	0.97	0.02
TMT Part A errors [visuospatial attention] [0-22]	0.17 (0.38)	0.33 (0.61)	0.25 (0.51)	0.34[b]	1.00	0.11
TMT Part B (sec) [visuospatial attention]	59.97 (22.11)	66.10 (25.13)	63.08 (23.69)	0.32[a]	1.00	0.26
TMT Part B errors [visuospatial attention] [0-24]	0.14 (0.35)	0.43 (1.28)	0.29 (0.95)	0.70[b]	1.00	0.04
HVLT - Total Recall [verbal memory] [0-36]	26.03 (4.75)	26.43 (5.76)	26.24 (5.25)	0.69[b]	1.00	0.06
HVLT - Delayed Recall [verbal memory] [0-12]	9.21 (2.02)	8.93 (2.32)	9.07 (2.16)	0.71[b]	1.00	0.06
HVLT – Recognition [verbal memory] [0-12]	11.31 (0.76)	11.40 (0.89)	11.36 (0.83)	0.63[b]	1.00	0.07

ROCF – Copy [visuospatial abilities] [0-36]	33.50 (3.58)	34.38 (1.75)	33.95 (2.81)	0.36[b]	1.00	0.13
ROCF - Immediate Recall [visual memory] [0-36]	18.12 (7.26)	17.67 (7.17)	17.89 (7.15)	0.79[b]	1.00	0.04
ROCF - Delayed Recall [visual memory] [0-36]	18.02 (6.86)	17.72 (6.42)	17.87 (6.59)	0.94[b]	0.97	0.01
ROCF – Recognition [visual memory] [0-24]	19.79 (2.66)	19.83 (1.91)	19.81 (2.29)	0.56[b]	1.00	0.09
VST - Egocentric Navigation [0-7]	2.59 (1.57)	2.60 (1.65)	2.59 (1.60)	0.95[b]	0.95	0.01
VST - Allocentric Navigation	17.09 (9.35)	13.65 (5.75)	15.34 (7.86)	0.19[b]	1.00	0.20
VST - Heading Direction [spatial navigation] [0-7]	5.28 (1.51)	5.80 (1.32)	5.54 (1.43)	0.19[b]	1.00	0.19

Table 17. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – Actigraphy session. Statistical tests and measures of effect sizes: a – independent sample t-test & Cohen's d), b – Mann-Whitney U Test & Rank-Biserial Correlation, c – Chi^2 & Cramer's V; Abbreviations: *CCI* - Cognitive Change Index; *PHQ-9* - Patient Health Questionnaire 9; *GAD-7* - General Anxiety Disorder 7; *PSQI* - Pittsburgh Sleep Quality Index; *ISI* - Insomnia Severity Index; *ESS* - Epworth Sleepiness Scale; *MEQ* – Morningness Eveningness Questionnaire; *m-ACE* - mini Addenbrooke's Cognitive Examination; *SDMT* - Symbol Digits Modalities Test; *TM* - Trail Making Test; *HVLT* - Hopkins Verbal Learning Test; *ROCF* - Rey Osterrieth Complex Figure; *VST* – Virtual Supermarket Task Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj)

6.5.2. Objective circadian rest-activity measures – ε4+ vs. ε4- allele carriers

While there were no genotype differences between actigraphy outcome measures, there was a trend toward significance for circadian amplitude (Relative Amplitude - ε4+(*M*±*SD*): 0.93±0.03; ε4-(*M*±*SD*): 0.95±0.03), and degree of stability in the rest-activity patterns, i.e., day-to-day consistency (Interdaily Stability - ε4+(*M*±*SD*): 0.48±0.14; ε4-(*M*±*SD*): 0.53±0.11) with lower values in ε4 carriers compared to controls (ε3/ε3) (Table 18, Figure 29A and 29B, respectively). Cohen's d indicated small effect sizes of APOE status for Interdaily Stability (*d*=0.37) and Activity-to-Rest probability (*d*=0.21), while medium for Relative Amplitude (*d*=0.50).

Dependent variable [range]	*APOE-ε4+ (N=28) (M (SD)*	*APOE-ε4- (N=30) (M (SD)*	*Total (N=58) (M (SD)*	*p*	*p-adj*	*Effect size*
Interdaily Stability [0-1]	0.48 (0.14)	0.53 (0.11)	0.51 (0.13)	0.17 [a]	0.68	0.37
Intra-Daily Variability [0-1]	0.90 (0.24)	0.88 (0.22)	0.89 (0.23)	0.74 [a]	1.00	0.09
Relative Amplitude (RA) [0-1]	0.93 (0.03)	0.95 (0.03)	0.94 (0.03)	0.06 [a]	0.48	0.50
Rest-to-Activity probability [0-1]	0.03 (0.02)	0.03 (0.01)	0.03 (0.01)	0.93 [a]	1.00	0.02
Activity-to-Rest probability [0-1]	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.43 [a]	1.00	0.21
Cosinor Amplitude	88.87 (40.68)	88.94 (29.20)	88.91 (34.66)	0.99 [a]	0.99	0.00
Cosinor Acrophase	4.65 (1.25)	4.63 (1.79)	4.64 (1.55)	0.97 [a]	1.00	0.01
Cosinor MESOR	89.17 (40.95)	91.68 (28.25)	90.51 (34.42)	0.79 [a]	1.00	0.07

Table 18. Group differences in actigraphy outcome measures between APOE ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: independent sample t-test & Cohen's *d*; Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).

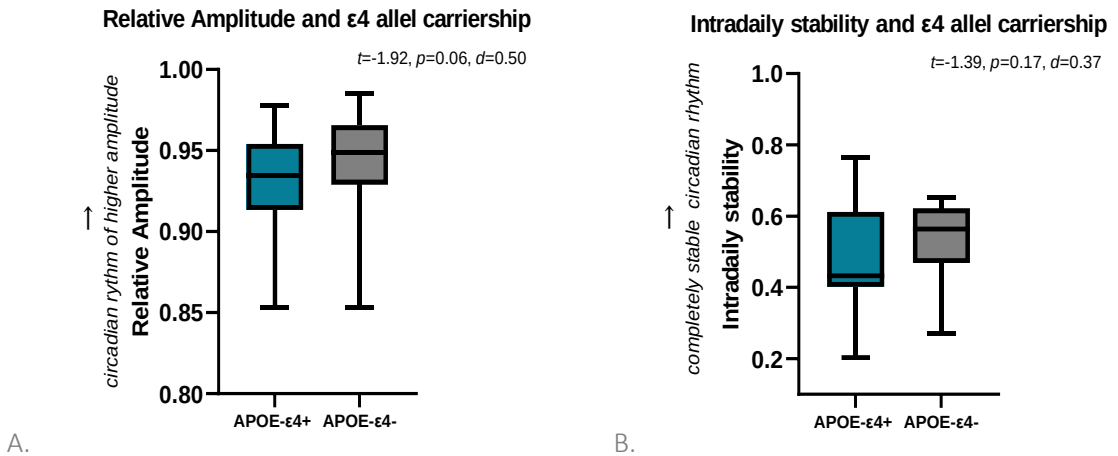


Figure 29. Differences in the Relative Amplitude of actigraphy measured circadian rest-activity rhythmicity(A) and Interdaily stability (B) between ε4 allele carriers (blue) and non-carriers (grey).

6.5.3. Does the APOE-ε4 allele affect actigraphy-assessed circadian rest-activity patterns in the habitual environment regardless of possible confounding factors such as age and biological sex?

ANCOVA including APOE-ε4 allele carriership, sex, and age as predictors was run for the actigraphy outcome measures (Table 19). While age and sex had multiple independent effects on actigraphy measures of circadian rest-activity rhythmicity, APOE-ε4 status independently predicted only circadian amplitude (Relative Amplitude). The Cosinor Acrophase residuals were not normally distributed and failed to be normalized using data transformations, hence ANCOVA was not conducted for this outcome measure.

Eta square effect size values for predictor *age* were the largest for the Cosinor Amplitude (η^2=0.14), Mesor (η^2=0.16) and Relative Amplitude (η^2=0.14), while for predictor *sex* the effect size estimates were largest for Interdaily Stability (η^2=0.12) and Interdaily Variability (η^2=0.08). For the predictors APOE-ε4 status, only Interdaily Stability (η^2=0.04) and Activity-to-Rest probability (η^2=0.01) reached small effect sizes at most, while Relative Amplitude presented a medium effect size (η^2=0.07).

Dependent variable	*Predictors*								
	age			sex			APOE-$\varepsilon4$ status		
	F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
Interdaily Stability	2.09	0.15	0.03	7.62	0.008	0.12	2.38	0.13	0.04
Intra-Daily Variability	0.72	0.40	0.01	4.73	0.03	0.08	0.23	0.64	0.00
Relative Amplitude	9.36	0.003	0.14	1.35	0.25	0.02	4.58	0.04	0.07
Rest-to-Activity probability	1.42	0.24	0.02	3.56	0.07	0.06	0.05	0.83	0.00
Activity-to-Rest probability (log10)	0.16	0.69	0.00	0.04	0.85	0.00	0.62	0.43	0.01
Cosinor Amplitude	8.48	0.005	0.14	0.09	0.77	0.00	0.03	0.86	0.00
Cosinor MESOR	10.24	0.002	0.16	0.10	0.76	0.00	0.24	0.63	0.00

Table 19. ANCOVA outcomes for actigraphy-based outcome measures. Statistical test and measure of effect size: ANCOVA & eta squared.

6.5.4. What is the relationship between the actigraphy-assessed circadian rest-activity rhythmicity in the habitual environment and spatial navigation and how is the association modulated by the APOE-ε4 allele carriership?

Spearman's rank correlation coefficient was used to assess the relationship between actigraphy-derived variables and spatial navigation (VST) (Figure 30). As shown in Table 20, better performance in the VST head direction task (VST-HD) and VST allocentric task (VST-A) was significantly associated with lower age. Better allocentric task execution was associated with higher circadian amplitude (Relative Amplitude and the Cosinor-based circadian amplitude). Additionally, better VST-A performance was significantly correlated with higher Cosinor MESOR and lower Interdaily Stability, i.e., less stable rest-activity circadian pattern. Note that significant associations ranged from negligible to small, with the only correlation between age and allocentric navigation reaching a moderate strength association (*rho*=0.59). Separate correlational plots for ε4+ and ε4- allele carriers can be found in Appendix 11 - *Associations between actigraphy-based rest-activity variables and the Supermarket task in APOE-ε4+ and non-carriers*.

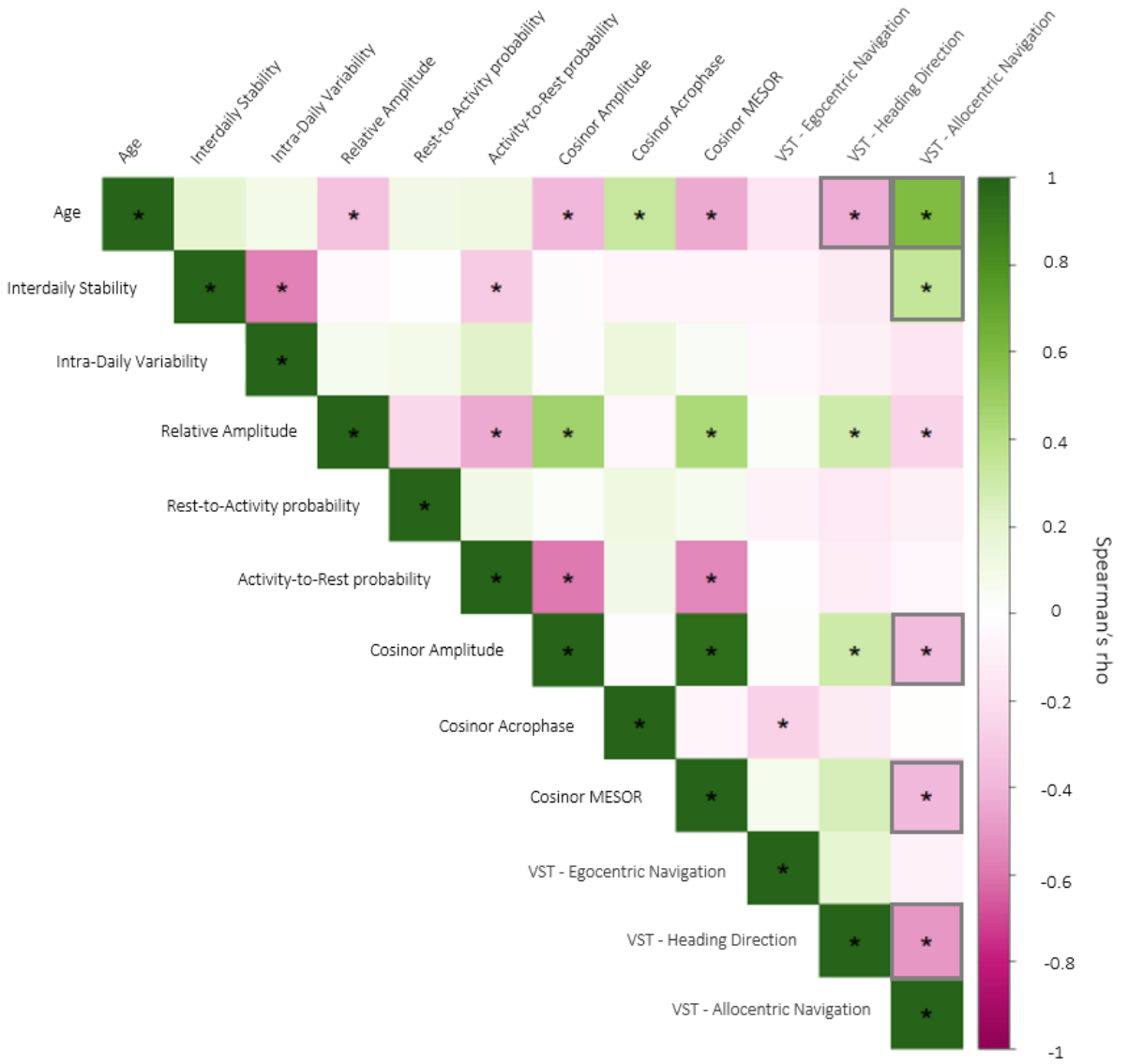


Figure 30. Adjacency matrix showing associations between actigraphy variables and the Virtual Supermarket task (n=58). Stars indicate significant correlations (p<0.05). The colour scale indicates the strength and the direction of the correlation. The further Spearman's ρ is from zero, the stronger the association between the variables. Significant VST correlations that survived Benjamini-Hochberg's correction are highlighted in grey.

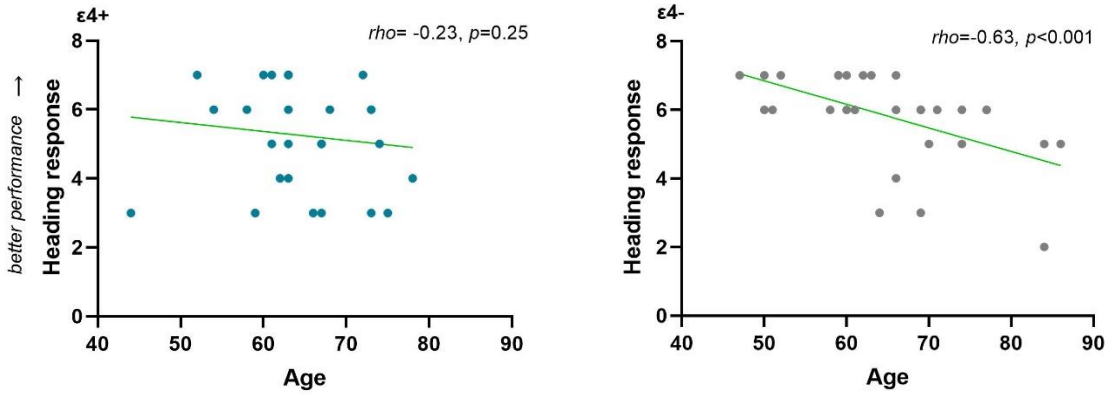
Variables	Egocentric navigation (VST-E)			Heading Direction (VST-HD)			Allocentric Direction (VST-A)		
	Spearman's rho	*p*	*p-adjusted*	*Spearman's rho*	*p*	*p-adjusted*	*Spearman's rho*	*p*	*p-adjusted*
age	-	-	-	-0.42	<0.001	0.01	0.59	<0.001	<0.001
Interdaily Stability	-	-	-	-	-	-	0.35	0.01	0.02
Relative Amplitude	-	-	-	0.30	0.02	0.07	-0.26	0.05	0.09
Cosinor Amplitude	-	-	-	0.31	0.02	0.08	-0.36	0.01	0.01
Cosinor Acrophase	-0.27	0.05	0.52	-	-	-	-	-	-
Cosinor Mesor	-	-	-	0.26	0.05	0.11	-0.39	< 0.001	0.01

Table 20. Significant correlations for Egocentric, Heading direction and Allocentric Virtual Supermarket task measures (shown in Figure 30). Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj) (all 11 variables were used as *i*, i.e., the total number of ranks).

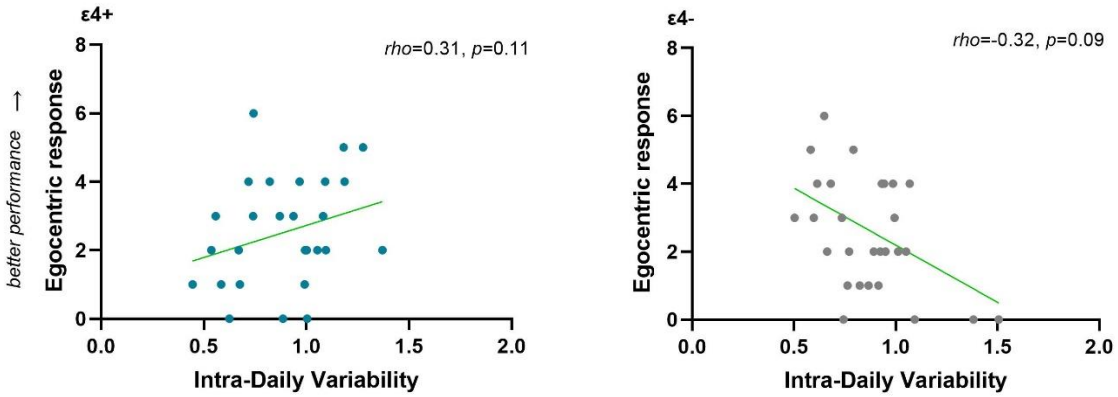
Additionally, Spearman's correlations involving actigraphy outcome measures and spatial navigation performance measured by the Supermarket tasks were run independently for APOE-ε4 allele carriers and non-carriers. The correlations were then compared using Fisher's *r*-to-*Z* transformation. Significant and trending towards significant differences were found for associations between age and Heading direction, Cosinor Acrophase and Heading direction, Interdaily stability and Heading direction, Activity to Rest probability and Heading direction, Cosinor Acrophase and Egocentric navigation, as well as Cosinor Amplitude and Allocentric response (Table 21). A better Heading response was associated with lower age (Figure 31 A), better Egocentric navigational performance was associated with lower Intra-daily variability, i.e., lower fragmentation of rest-activity patterns (Figure 31 B), while a better Allocentric response with higher circadian amplitude (Cosinor Amplitude) (Figure 31 D). Further, higher Cosinor Acrophase, i.e., the timing of the highest activity was negatively correlated with better Egocentric and Heading responses (Figures 31 E and F). Importantly, all the listed associations were significant *only* for controls (ε3/ε3). The association between Activity-to-Rest probability was neither significant for at-risk participants nor the controls (ε3/ε3) (Figure 31 C).

Outcome measures	Egocentric Navigation (VST-E)			Heading Direction (VST-HD)			Allocentric Navigation (VST-A)		
	z	*p*	*p*-adj	*z*	*p*	*p*-adj	*z*	*p*	*p*-adj
Age	0.41	0.68	0.83	1.83	0.07	0.39	-0.41	0.68	0.99
Interdaily Stability	-0.12	0.90	0.90	0.74	0.46	0.72	0.39	0.70	1.00
Intra-Daily Variability	2.34	0.02	0.22	0.86	0.39	1.07	-0.29	0.77	1.00
Relative Amplitude (RA)	-1.02	0.31	0.68	-0.60	0.55	0.76	0.00	1.00	1.00
Rest-to-Activity probability	1.47	0.14	0.39	0.79	0.43	0.79	0.37	0.71	1.00
Activity-to-Rest probability	1.72	0.09	0.33	-0.04	0.97	0.97	-0.43	0.67	1.00
Cosinor Amplitude	-0.72	0.47	0.65	-1.16	0.25	0.92	1.70	0.09	1.00
Cosinor Acrophase	2.34	0.02	0.11	2.12	0.04	0.44	-0.22	0.83	0.91
Cosinor MESOR	-0.77	0.44	0.69	-0.86	0.39	0.86	1.37	0.17	0.94
VST - Egocentric Navigation	-	-	-	-0.31	0.76	0.93	-0.88	0.38	1.00
VST – Heading direction	-0.31	0.76	0.84	-	-	-	-0.25	0.81	0.99
VST - Allocentric Navigation	-0.88	0.38	0.70	-0.24	0.81	0.89	-	-	-

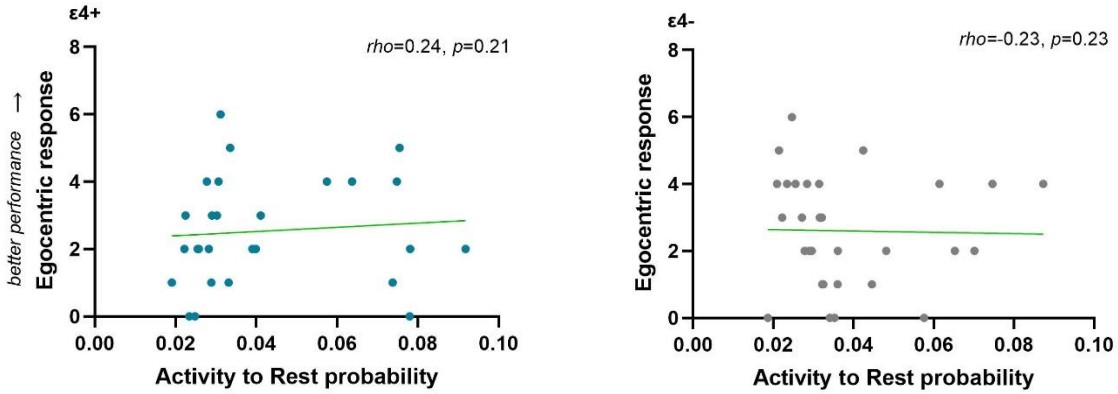
Table 21. Comparison of Spearman's rho correlation coefficients between APOE ε4 allele carriers and non-carriers involving actigraphy outcome measures. Statistical test and measure of effect size: Fisher 1925. Benjamini-Hochberg procedure was applied to calculate adjusted *p*-values (*p*-adj).


ε4+
rho= -0.23, p=0.25
better performance
Heading response
Age
ε4-
rho=-0.63, p<0.001
Heading response
Age

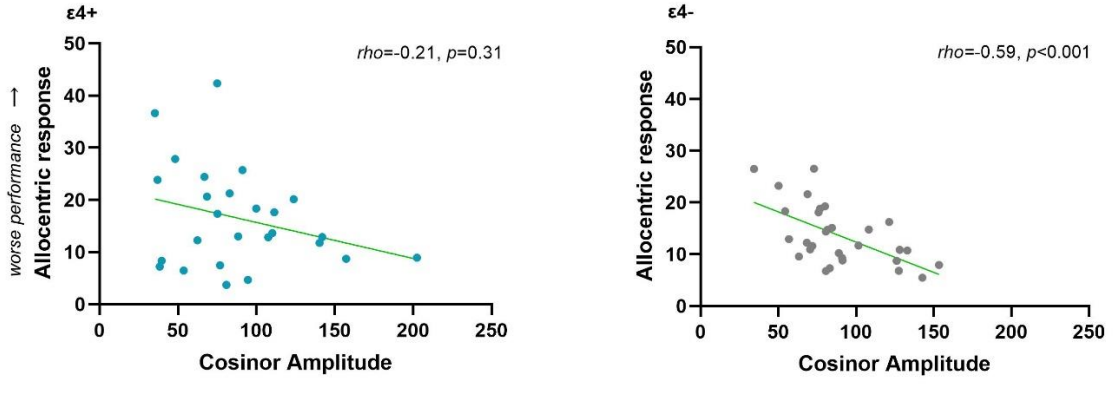
A.


ε4+
rho=0.31, p=0.11
better performance
Egocentric response
Intra-Daily Variability
ε4-
rho=-0.32, p=0.09
Egocentric response
Intra-Daily Variability

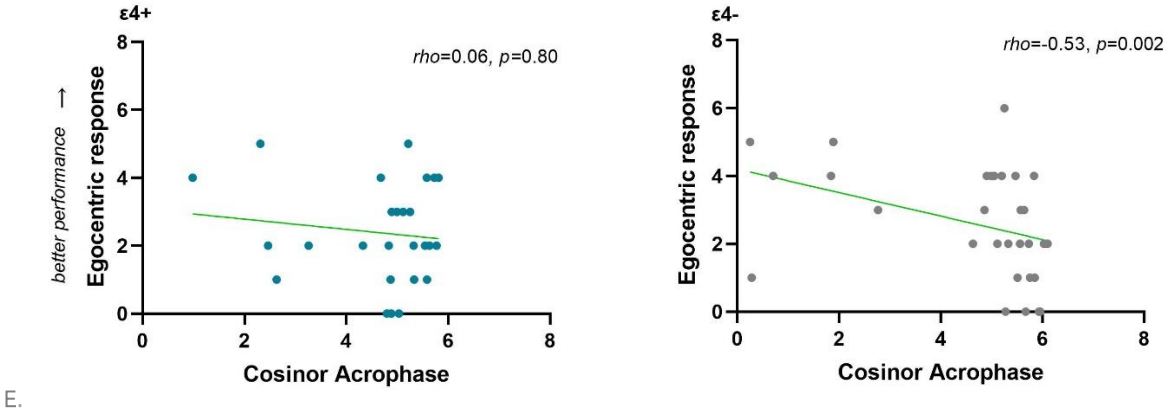
B.


ε4+
rho=0.24, p=0.21
better performance
Egocentric response
Activity to Rest probability
ε4-
rho=-0.23, p=0.23
Egocentric response
Activity to Rest probability

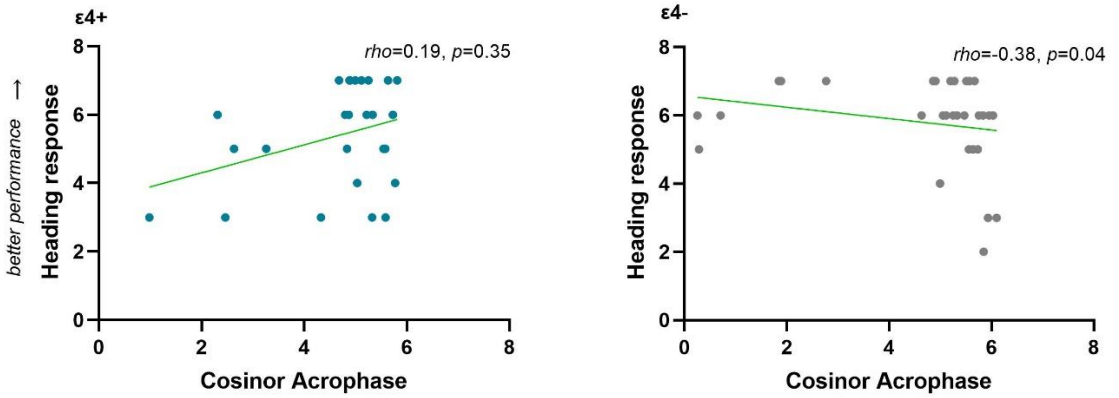
C.


ε4+
rho=-0.21, p=0.31
worse performance
Allocentric response
Cosinor Amplitude
ε4-
rho=-0.59, p<0.001
Allocentric response
Cosinor Amplitude

D.



E.



F.

Figure 31. The associations – Table 21. Association between Heading Response and age (A), Intra-daily Variability and Egocentric Response (B), Activity to Rest and Egocentric Response (C), Cosinor Amplitude and Egocentric Response (D), Cosinor Acrophase and Egocentric Response (E) and Cosinor Acrophase and Heading Response (F) in ε4 allele carriers and non-carriers. The regression lines shall not be interpreted in the context of reported Spearman rho as they are used solely for visualizing the direction of the association.

Based on the results indicating that better allocentric response is positively associated with higher Circadian Amplitude (Cosinor Amplitude) (Figure 31D), additional analyses addressing the functional implications of this relationship were run. The multivariate regression model[68] returned a significant independent effect of age ($p \leq 0.001$) and sex ($p \leq 0.001$) on the allocentric response. Cosinor Amplitude and genotype yielded a trend toward significance ($p=0.058$, $p=0.051$, respectively). The model ($F=11.99$, $p \leq 0.0001$) explained 44% of the variance in the Allocentric response ($R^2=0.44$). Further, based on the strong correlation between allocentric navigation and visuospatial memory (Figure 27, Table 15), the second model was run with an additional cognitive predictor, i.e., ROCF – Immediate Recall. The addition increased the R^2 to 51%. Model two ($F=12.25$, $p \leq 0.0001$) revealed a significant independent effect of age ($p=0.01$), sex ($p=-0.001$), genotype ($p=0.03$) and visuospatial memory ($p=0.01$), while Cosinor Amplitude ($p=0.08$) was trending towards significance. Further, a simple linear

[68] lm(allocentric performance ~ age + sex + APOE-e4 status + Cosinor Amplitude + ROCF-Immediate Recall

model between allocentric response and Cosinor amplitude was performed revealing R^2 =0.13 (F=9.40, p=0.003).

6.5.5. Subjective sleep measures – ε4+ vs. ε4- allele carriers

There were no genotype differences in any of the sleep diary outcome measures as analysed by an independent samples t-test (Table 22). The meaningful, small effect sizes were found for the level of strenuous activity (d=0.35) and averaged time in bed (d=0.24).

Outcome measures [range]	*APOE-ε4+ (N=29) (M (SD)*	*APOE-ε4- (N=29) (M (SD)*	*Total (N=58) (M (SD)*	*p*	*p-adj*	*Effect size*
Length of recording [days]	14.03 (0.78)	14.03 (1.64)	14.03 (1.27)	1.00	1.00	-
Average TIB [min]	548.00 (53.30)	533.15 (69.42)	540.84 (61.49)	0.37	1.00	0.24
Average TST [min]	447.21 (58.48)	443.29 (51.91)	445.28 (54.89)	0.79	1.00	0.07
Average Sleep Latency [min]	15.41 (10.94)	15.21 (10.60)	15.32 (10.68)	0.95	1.00	0.02
average number of awakenings	2.01 (1.24)	2.14 (1.53)	2.08 (1.38)	0.73	1.00	0.09
Average length of awakenings [min]	22.79 (18.11)	21.93 (17.98)	22.36 (17.89)	0.86	1.00	0.05
Average SE [logit]	0.72 (0.26)	0.72 (0.20)	0.72 (0.23)	0.96	1.00	0.01
Averaged midpoint of sleep	03:47 AM	03:46 AM	03:46 AM	-	-	-
Average Self-rated Quality of Sleep [1-9]	4.38 (1.12)	4.36 (1.18)	4.37 (1.14)	0.96	1.00	0.01
Average KSS Evening [1-9]	5.53 (1.49)	5.32 (1.70)	5.42 (1.59)	0.63	1.00	0.13
Average KSS Morning [1-9]	4.28 (1.27)	4.15 (1.39)	4.22 (1.32)	0.73	1.00	0.07
Average level of strenuous activity	0.29 (0.25)	0.20 (0.28)	0.25 (0.27)	0.21	1.00	0.35
Average use of Alarm Clock to wake up	0.15 (0.26)	0.20 (0.31)	0.17 (0.29)	0.51	1.00	0.18
Average level of difficulty to get up [1-9]	3.77 (1.60)	3.61 (1.24)	3.69 (1.42)	0.68	1.00	0.11
Average dream recall	0.28 (0.31)	0.26 (0.27)	0.27 (0.29)	0.78	1.00	0.08

Table 22. Group differences in sleep diary outcome measures between APOE ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: independent sample t-test & Cohen's *d*; Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).

6.5.6. Does the APOE-ε4 allele affect self-reported sleep quality and sleep efficiency as measured by sleep diary in the habitual environment regardless of possible confounding factors such as age and biological sex?

ANCOVA with APOE-ε4 allele carriership, sex, and age as predictors was run for sleep-diary-based self-reported sleep measures. The APOE-ε4 allele has not reached a significance level for any of investigated variables while age and sex did significantly predict some of the outcome measures (Table 23). For predictor age, the biggest effect sizes were found for morning and evening sleepiness assessed by KSS (η^2=0.09, η^2=0.06, respectively), dream recall (η^2=0.07) and sleep latency (η^2=0.06), while time

in bed (η^2=0.07) and level of difficulties to get up on the morning (η^2=0.08) for predictor sex. APOE-ε4 status' effect sizes were insignificant besides small effects for total time in bed (η^2=0.01) and level of strenuous activities (η^2=0.03).

Dependent variable	*Predictors*								
	age			sex			APOE- $\varepsilon 4$ status		
	F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
Average TIB [min]	2.46	0.12	0.04	4.28	0.04	0.07	0.76	0.39	0.01
Average TST [min]	0.53	0.47	0.01	1.50	0.23	0.03	0.06	0.81	0.00
Average Sleep Latency [min]	3.48	0.07	0.06	1.19	0.28	0.02	0.02	0.91	0.00
Average number of awakenings	0.31	0.58	0.00	0.02	0.88	0.00	0.14	0.71	0.00
Average length of awakenings [min]	0.35	0.56	0.01	0.00	0.97	0.00	0.05	0.83	0.00
Average SE [logit]	2.51	0.12	0.05	0.39	0.54	0.01	0.00	0.99	0.00
Average Self-reported Quality of Sleep	0.04	0.84	0.00	0.72	0.40	0.01	0.00	1.00	0.00
Average KSS Evening	3.22	0.08	0.06	0.22	0.64	0.00	0.10	0.75	0.00
Average KSS Morning	4.85	0.03	0.09	0.20	0.66	0.00	0.02	0.88	0.00
Average level of strenuous activity	0.98	0.33	0.02	0.27	0.61	0.01	1.57	0.22	0.03
Average use of Alarm Clock to wake up	0.09	0.76	0.00	0.94	0.34	0.02	0.45	0.51	0.00
Average level of difficulty to get up	1.19	0.28	0.02	4.31	0.04	0.08	0.08	0.78	0.00
Average dream recall	3.86	0.06	0.07	0.17	0.68	0.00	0.02	0.90	0.00

Table 23. ANCOVA outcomes for sleep diary-based outcome measures. Statistical test and measure of effect size: ANCOVA & eta squared.

6.5.7. What is the association of subjective sleep quality as measured by sleep diary in the habitual environment with spatial navigation and how is the association modulated by the APOE-ε4 allele carriership?

Spearman's rank correlation coefficient was used to assess the relationship between sleep diary variables and spatial navigation (VST) (Figure 32). Spearman's rho test yielded significance for the negative correlation between Heading response and level of difficulty to get up (*rho*=-0.32, *p*=0.02), i.e., worse spatial navigation performance is associated with more severe problems getting up in the morning. Heading direction performance was strongly positively correlated with allocentric performance (rho=-0.49, *p*<0.001) indicating that the two outcome measures assess a similar underlying construct of spatial navigation performance in the studied sample. Correlational plots for ε4+ and ε4- allele carriers can be found in Appendix 12 - *Associations between sleep diary-based outcome measures and the Supermarket task in APOE-ε4+ and non-carriers*.

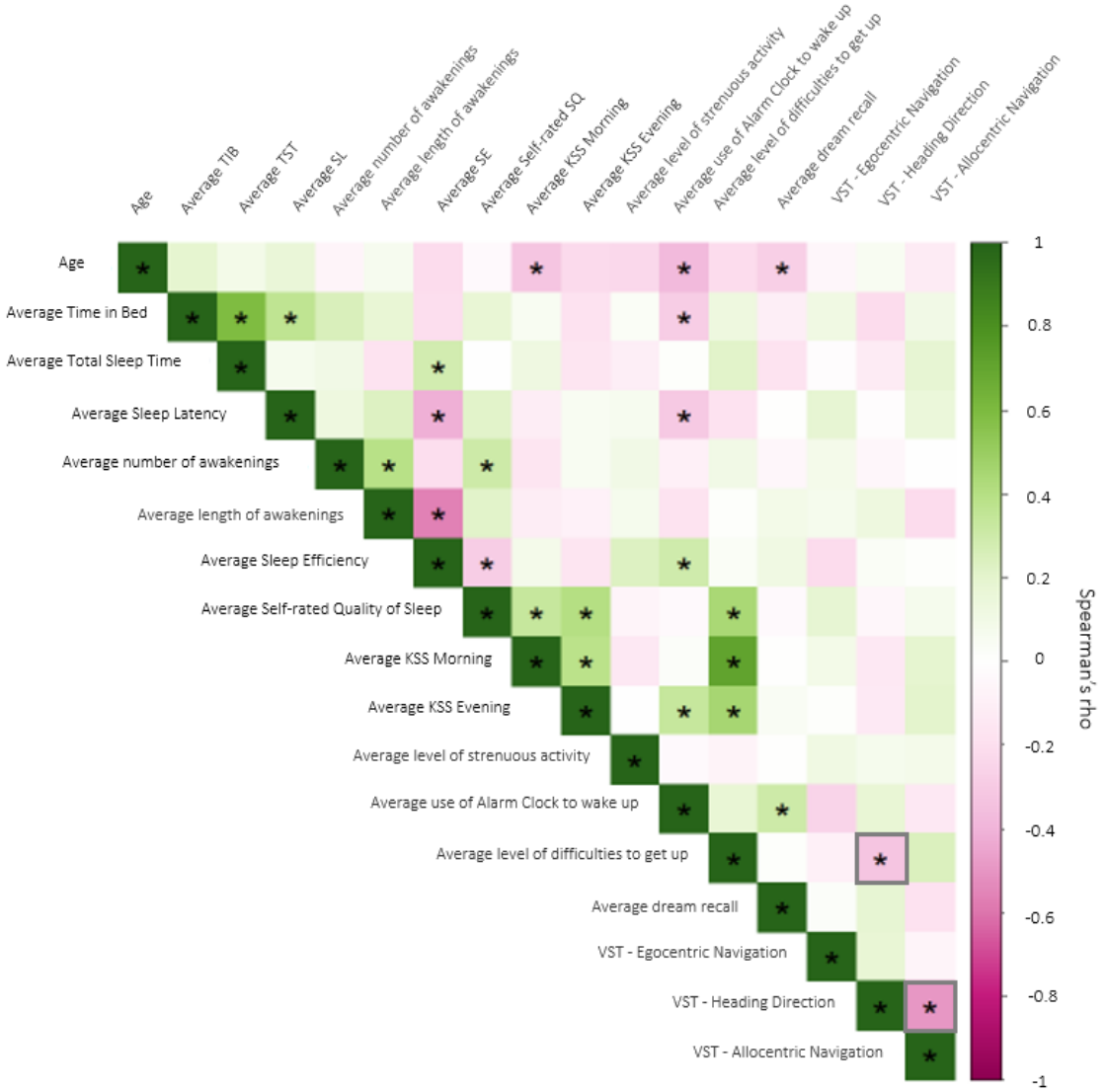


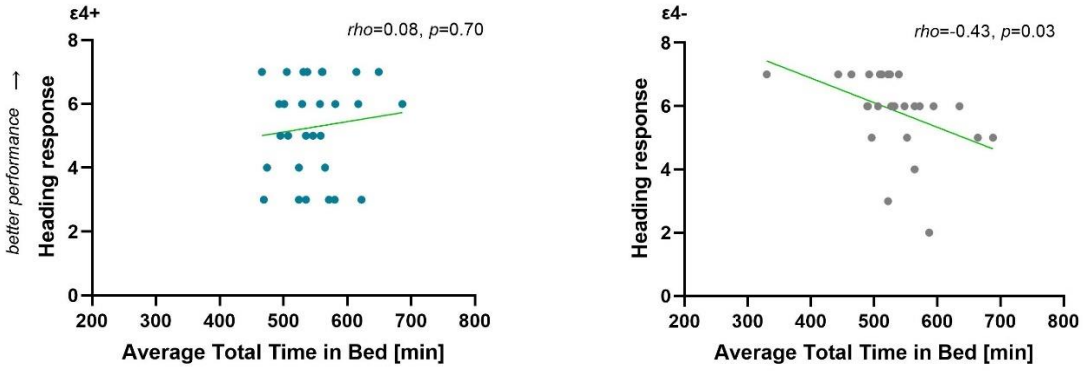
Figure 32. Adjacency matrix displaying associations between sleep diary variables and spatial navigation – Virtual Supermarket task (n=58). Stars indicate significant correlations (p<0.05). The colour scale indicates the strength and direction of the correlation. The further Spearman's ρ is from zero, the stronger the association between the variables. The sign of ρ indicates the direction of the relationship. Significant VST correlations that survived Benjamini-Hochberg's correction are highlighted in grey.

When comparing, the correlation coefficients of APOE-ε4 allele carriers and non-carriers using the Fisher *r*-to-*z* test, several outcome measures revealed significant genotype differences (Table 24). Additional analysis showed that better Heading response was significantly correlated with less time spent in bed in controls (ε3/ε3) only, (Figure 33 A), while in at-risk participants (ε4+), better allocentric navigation was correlated with longer time spent awake during the night (Figure 33 D). Further, in at-risk risk gene carriers but not in controls (ε3/ε3), lower self-reported level of sleepiness in the morning was associated with better allocentric and Heading responses (KSS, Figure 33 E and F) and lower level of evening sleepiness (KSS evening) were correlated with better Heading performance (Figure 33 G). Similarly increased difficulties to get up in the morning were associated with poorer allocentric and

heading responses in the elevated risk group (Figure 33 J and K). The strength of correlations (for significant associations) ranges from low to moderate with the largest size of correlation being found for heading direction x difficulties to get up (*rho*=-0.59) and heading direction x morning sleepiness (*rho*=-0.50).

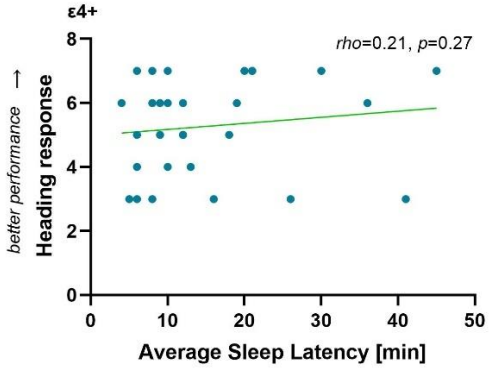
Outcome measures	Egocentric Navigation (VST-E)			Heading Direction (VST-HD)			Allocentric Navigation (VST-A)		
	z	*p*	*p*-adj	*z*	*p*	*p*-adj	*z*	*p*	*p*-adj
Age	0.53	0.60	1.00	2.98	0.003	0.02	-1.12	0.26	0.69
Average TIB [min]	-1.28	0.20	1.00	2.52	0.01	0.05	-1.01	0.31	0.62
Average TST [min]	0.29	0.77	1.00	1.26	0.21	0.48	-0.69	0.45	0.60
Average Sleep Latency [min]	1.30	0.19	1.00	1.96	0.05	0.13	-0.85	0.40	0.71
Average number of awakenings	0.79	0.43	1.00	0.99	0.32	0.57	-2.09	0.04	0.64
Average length of awakenings [min]	-0.22	0.83	1.00	0.17	0.86	0.98	-1.90	0.06	0.32
Average SE [logit]	-0.37	0.71	1.00	-0.72	0.47	0.75	1.10	0.27	0.62
Average Self-reported Quality of Sleep	0.14	0.89	1.00	0.06	0.95	0.95	0.14	0.89	1.00
Average KSS Morning	-0.66	0.51	1.00	-3.04	0.002	0.03	1.85	0.06	0.24
Average KSS Evening	0.01	1.00	1.00	-2.28	0.02	0.06	0.77	0.44	0.64
Average level of strenuous activity	0.59	0.56	1.00	-0.35	0.73	0.97	0.06	0.95	1.00
Average use of Alarm Clock to wake up	0.03	0.97	1.00	-1.24	0.21	0.42	1.69	0.09	0.29
Average level of difficulty to get up	0.15	0.88	1.00	-2.76	0.01	0.04	2.02	0.04	0.32
Average dream recall	-0.10	0.92	1.00	-0.46	0.64	0.93	0.00	1.0	1.00
VST - Egocentric Navigation	-	-	-	-0.25	0.80	0.98	-0.80	0.42	0.67
VST – Heading direction	-0.25	0.80	1.00	-	-	-	-0.16	0.88	1.00
VST - Allocentric Navigation	-0.80	0.42	1.00	-0.16	0.88	0.94	-	-	-

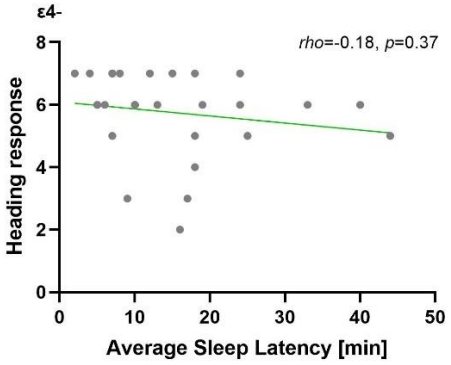
Table 24. Comparison of Spearman's rho correlation coefficients between ε4 allele carriers and non-carriers involving sleep diary outcome measures. Statistical test and measure of effect size: Fisher 1925. Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).



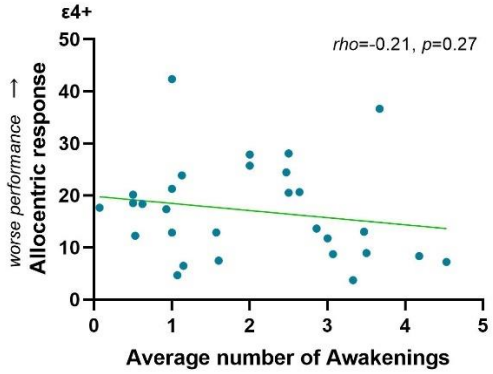
A.

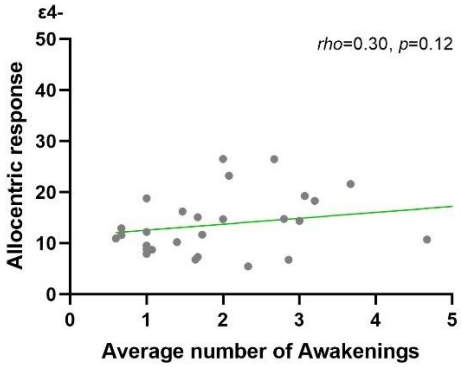
B.


ε4+
rho=0.21, p=0.27
better performance
Heading response
Average Sleep Latency [min]

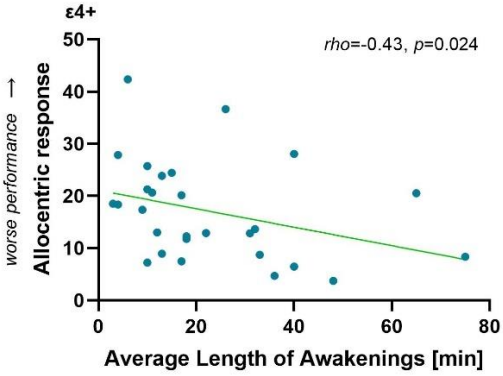

ε4-
rho=-0.18, p=0.37
Heading response
Average Sleep Latency [min]

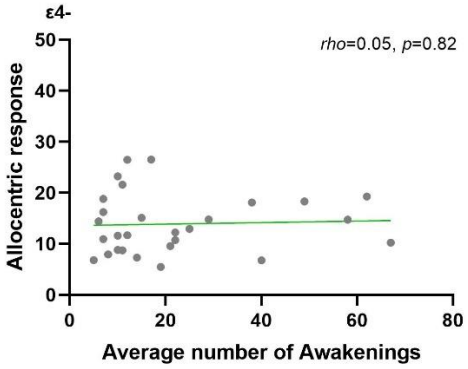
C.


ε4+
rho=-0.21, p=0.27
worse performance
Allocentric response
Average number of Awakenings

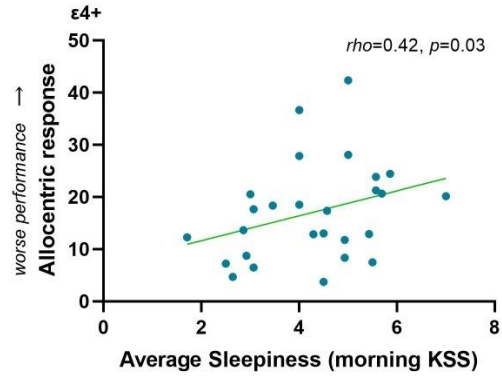

ε4-
rho=0.30, p=0.12
Allocentric response
Average number of Awakenings

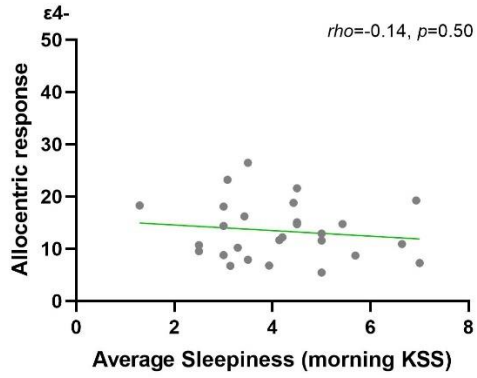
D.


ε4+
rho=-0.43, p=0.024
worse performance
Allocentric response
Average Length of Awakenings [min]

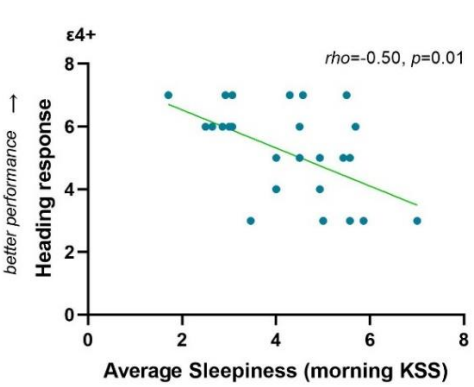

ε4-
rho=0.05, p=0.82
Allocentric response
Average number of Awakenings

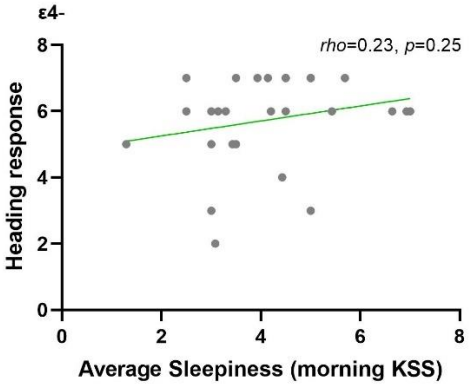
E.


ε4+
rho=0.42, p=0.03
worse performance
Allocentric response
Average Sleepiness (morning KSS)

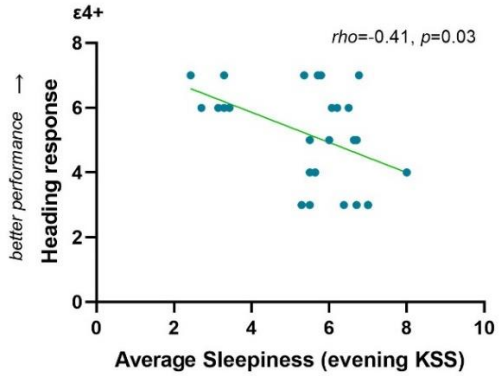

ε4-
rho=-0.14, p=0.50
Allocentric response
Average Sleepiness (morning KSS)

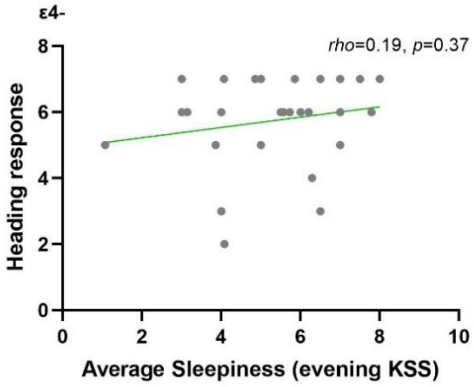
F.


ε4+
rho=-0.50, p=0.01
better performance
Heading response
Average Sleepiness (morning KSS)

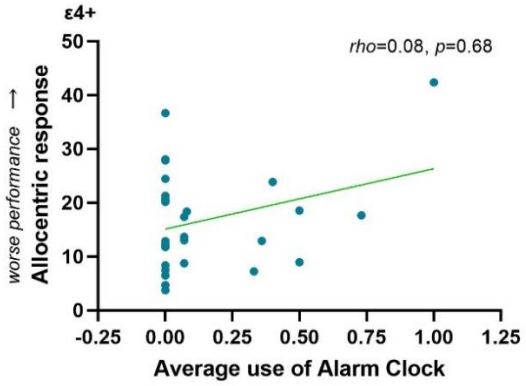

ε4-
rho=0.23, p=0.25
Heading response
Average Sleepiness (morning KSS)

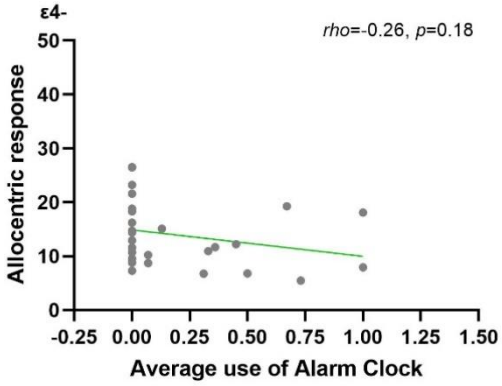
G.


ε4+
rho=-0.41, p=0.03
better performance
Heading response
Average Sleepiness (evening KSS)

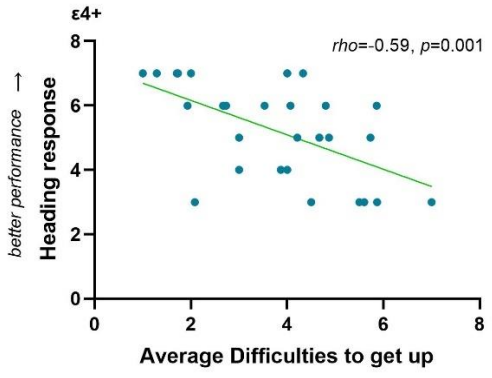

ε4-
rho=0.19, p=0.37
Heading response
Average Sleepiness (evening KSS)

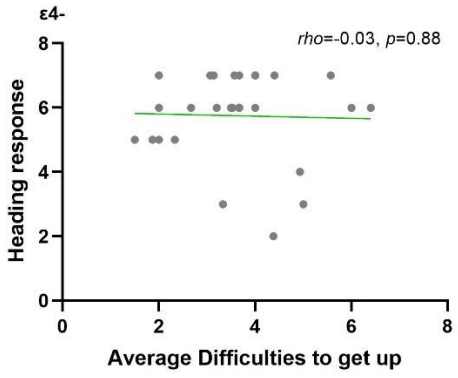
H.

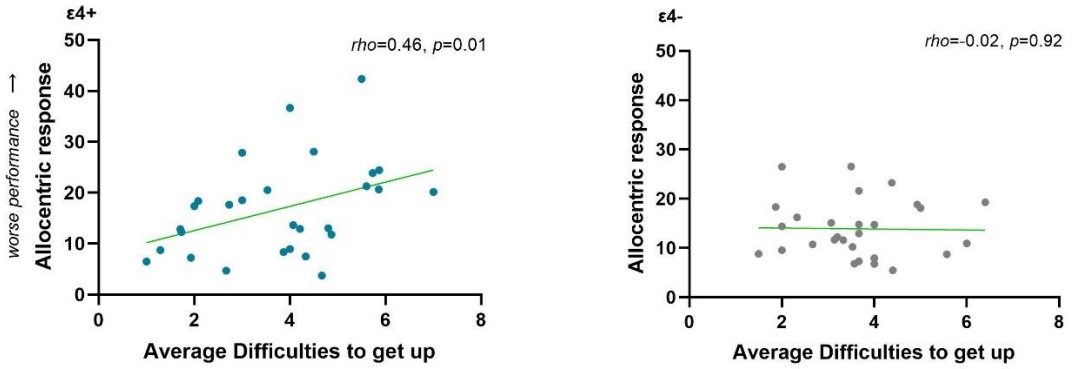

ε4+
rho=0.08, p=0.68
worse performance
Allocentric response
Average use of Alarm Clock


ε4-
rho=-0.26, p=0.18
Allocentric response
Average use of Alarm Clock

I.


ε4+
rho=-0.59, p=0.001
better performance
Heading response
Average Difficulties to get up


ε4-
rho=-0.03, p=0.88
Heading response
Average Difficulties to get up



J.

Figure 33. The associations – Table 24. Associations between Average Total time in Bed and Heading Response (A), Average sleep latency and Heading Response (B), Average number of awakenings and Allocentric Response (C), Average Length of awakenings and Allocentric Response (D) Average KSS Morning and Allocentric response (E) Average KSS Morning and Heading response (F) average KSS Evening and Heading Response (G), Average use of Alarm Clock and Allocentric response (H), Average Difficulties to get up and Heading response (I), Average Difficulties to get up and Allocentric Response (J), in ε4 allele carriers and non-carriers. The regression lines shall not be interpreted in the context of reported Spearman rho as they are used solely for showing trends.

SUMMARY of FINDINGS – Actigraphy (field) session

Analysis of the actigraphy-based rest-activity patterns revealed no significant APOE-ε4 status effects except a decrease in circadian Relative Amplitude and marginally lower stability of activity-rest patterns (Interdaily Stability) in APOE-ε4 allele carriers compared to non-carriers. No effects of APOE-ε4 allele status on self-reported sleep quality and sleep efficiency were found when analysing sleep diary outcome measures.

Visual Supermarket Task (VST) – heading and allocentric performance showed associations with Interdaily Stability and relative amplitude. The better allocentric and heading direction was, the higher circadian amplitude, while worse allocentric navigation was also related to higher Interdaily Stability and lower MESOR. APOE-ε4 allele status was shown to modulate the associations between actigraphy-based rest-activity patterns and spatial navigation performance. Better egocentric navigational performance (VST-E) was associated with lower fragmentation of rest-activity patterns (lower IV), while better allocentric response (VST-A) was correlated with higher circadian amplitude (Cosinor Amplitude). Additionally, the earlier timing of the highest circadian activity (lower Cosinor Acrophase) was associated with more efficient egocentric and heading responses (VST-E, VST-HD). Importantly, all the associations were significant only for APOE-ε4 allele non-carriers.

Sleep diary-assessed sleep measures showed several associations with spatial navigation and APOE-ε4 allele carriership. Higher average sleepiness in the morning (KSS) was associated with worse allocentric response (VST-A), while a lower morning and evening level of sleepiness (KSS) was correlated with better heading response (VST-HD). Further, more severe difficulties to get up in the morning were kindred with worse allocentric (VST-A) and heading responses (VST-HD). These effects were found only in at-risk participants (ε4 allele carriers). Moreover, two significant associations revealed unexpected directions, i.e., in controls (ε3/ε3), shorter total time in bed (TIB) was shown to be correlated with better heading direction response (VST-HD), while in the at-risk group (ε4+), longer nocturnal awakenings were associated with better allocentric response (VST-A).

6.6. Lab session

6.6.1. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – ε4+ vs. ε4- allele carriers

There were no significant differences between ε4 allele carriers and non-carriers in any of the demographic, psychological, sleep, sleepiness, chronotype or cognitive outcome measures besides VST – Heading Direction, where controls (ε3/ε3) performed significantly better than the ε4 allele carriers (ε4+(*M*±*SD*): 4.74±1.56, ε4-(*M*±*SD*): 6.22±0.73) (Table 25). Note that the effect has not survived multiple testing. For participants enrolled in the sleep lab session, the largest effect size was obtained for heading direction (VST, *d*=0.54), while the rest of the Cohen's *d* values oscillated between none to small.

Outcome measures [range]	*APOE-ε4+ (N=19) (M (SD)*	*APOE-ε4- (N=18) (M (SD)*	*Total (N=37) (M (SD)*	*p*	*p-adj*	*Effect size*
Age [years]	64.21 (8.58)	65.00 (9.54)	64.59 (8.94)	0.79[a]	0.99	0.09
Sex [female N[%]]	10 (52.63%)	10 (55.56%)	20 (54.05%)	0.86[c]	0.92	0.03
Years of education	17.32 (6.94)	15.56 (2.18)	16.46 (5.21)	0.69[b]	0.94	0.08
Self-reported hours of sleep	7.24 (0.65)	7.19 (0.66)	7.21 (0.64)	0.83[a]	0.96	0.08
PSQI [sleep quality] [0-21]	3.95 (2.22)	4.22 (1.93)	4.08 (2.06)	0.68[b]	0.97	0.08
ESS [sleepiness] [0-24]	4.16 (2.48)	4.11 (2.30)	4.14 (2.36)	0.95[b]	0.95	0.02
ISI [insomnia] [0-28]	2.89 (2.64)	2.78 (3.39)	2.84 (2.99)	0.51[b]	0.90	0.13
MEQ [chronotype] [16-86]	59.37 (7.89)	61.89 (7.98)	60.59 (7.92)	0.25[b]	0.94	0.23
PHQ-9 [depression] [0-27]	0.79 (1.18)	0.28 (0.67)	0.54 (0.99)	0.11[b]	1.00	0.25
GAD-7 [anxiety] [0-21]	0.47 (0.96)	0.72 (1.07)	0.59 (1.01)	0.33[b]	0.90	0.16
CCI total [subjective cognitive decline] [20-100]	28.47 (4.23)	28.83 (8.23)	28.65 (6.40)	0.45[b]	0.90	0.15
CCI memory [12-60]	18.84 (3.79)	19.06 (5.53)	18.95 (4.65)	0.70[b]	0.91	0.08
CCI executive functions [5-25]	5.47 (1.43)	5.06 (1.80)	5.27 (1.61)	0.13[b]	1.00	0.28
CCI language [3-15]	3.68 (0.75)	3.72 (1.41)	3.70 (1.10)	0.52[b]	0.87	0.11
m-ACE [global cognitive status] [0-30]	28.68 (1.38)	28.67 (1.24)	28.68 (1.29)	0.84[b]	0.93	0.04
SDMT [speed of processing] [0-110]	47.58 (5.26)	49.22 (8.85)	48.38 (7.18)	0.25[b]	0.83	0.22
TMT Part A (sec) [visuospatial attention]	28.16 (5.92)	28.39 (9.54)	28.27 (7.78)	0.93[a]	0.96	0.03
TMT Part A errors [visuospatial attention] [0-22]	0.16 (0.37)	0.39 (0.61)	0.27 (0.51)	0.21[b]	1.00	0.18
TMT Part B (sec) [visuospatial attention]	64.37 (25.10)	66.28 (26.03)	65.30 (25.22)	0.82[a]	0.98	0.08
TMT Part B errors [visuospatial attention] [0-24]	0.16 (0.37)	0.67 (1.61)	0.41 (1.17)	0.55[b]	0.87	0.08
HVLT - Total Recall [verbal memory] [0-36]	25.00 (5.79)	27.71 (5.06)	26.24 (5.56)	0.42[a]	0.97	0.16
HVLT - Delayed Recall [verbal memory] [0-12]	8.95 (2.17)	9.11 (2.35)	9.03 (2.23)	0.66[b]	0.99	0.08
HVLT – Recognition [verbal memory] [0-12]	11.15 (0.88)	11.47 (0.72)	11.30 (0.81)	0.19[a]	1.00	0.24

ROCF – Copy [visuospatial abilities] [0-36]	33.29 (4.16)	34.58 (1.91)	33.92 (3.29)	0.19[b]	1.00	0.25
ROCF - Immediate Recall [visual memory] [0-36]	16.63 (7.21)	18.83 (6.56)	17.70 (6.90)	0.47[b]	0.88	0.14
ROCF - Delayed Recall [visual memory] [0-36]	16.87 (6.99)	18.47 (5.51)	17.65 (6.28)	0.44[b]	0.94	0.15
ROCF – Recognition [visual memory] [0-24]	20.05 (2.48)	19.50 (1.86)	19.78 (2.19)	0.22[b]	0.94	0.23
VST - Egocentric Navigation [0-7]	2.68 (1.53)	3.11 (1.57)	2.89 (1.54)	0.36[b]	0.90	0.18
VST - Allocentric Navigation	18.19 (10.57)	13.65 (5.44)	15.98 (8.67)	0.25[b]	0.75	0.23
VST - Heading Direction [spatial navigation][0-7]	4.74 (1.56)	6.22 (0.73)	5.46 (1.43)	0.004[b]	0.12	0.54

Table 25. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – APOE-ε4 allele carriers vs controls (ε3/ε3) – Sleep lab session. Statistical tests and measures of effect sizes: a – independent sample t-test & Cohen's d), b – Mann-Whitney U Test & Rank-Biserial Correlation, c – Chi^2 & Cramer's V; Abbreviations: *CCI* - Cognitive Change Index; *PHQ-9* - Patient Health Questionnaire 9; *GAD-7* - General Anxiety Disorder 7; *PSQI* - Pittsburgh Sleep Quality Index; *ISI* - Insomnia Severity Index; *ESS* - Epworth Sleepiness Scale; *MEQ* – Morningness Eveningness Questionnaire; *m-ACE* - mini Addenbrooke's Cognitive Examination; *SDMT* - Symbol Digits Modalities Test; *TM* - Trail Making Test; *HVLT* - Hopkins Verbal Learning Test; *ROCF* - Rey Osterrieth Complex Figure; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj).

6.6.2. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – by protocol

Individuals who were *randomly* assigned to the SD protocol scored lower in CCI total (SD: 27±5.10; MN: 30.59±7.35), CCI language (SD(*M±SD*):3.35±0.49; MN(*M±SD*):4.12±1.45) and performed worse in HVLT – Delayed Recall (SD(*M±SD*): 8.30±2.43; MN(*M±SD*): 9.88±1.65) and Recognition (SD(*M±SD*):10.20±1.47; MN(*M±SD*):11.53±0.70) compared to the participants who underwent MN protocol (Table 26). The effect sizes ranged from negligible to small.

Outcome measures [range]	*Sleep deprivation (N=20) (M (SD)*	*Multinap (N=17) (M (SD)*	*Total (N=37) (M (SD)*	*p*	*p*-adj	*Effect size*
Age [years]	65.35 (9.87)	63.71 (7.91)	64.59 (8.94)	0.59[a]	0.90	0.12
Sex [female N[%]]	11 (55%)	9 (53%)	20 (54%)	0.90[c]		0.01
Years of education	15.63 (3.24)	16.00 (3.69)	15.81 (3.41)	0.75[a]	0.87	0.11
Self-reported hours of sleep	7.25 (0.65)	7.18 (0.66)	7.21 (0.64)	0.74[a]	0.93	0.11
PSQI [sleep quality] [0-21]	4.00 (2.25)	4.18 (1.88)	4.08 (2.06)	0.75[b]	0.84	0.07
ESS [sleepiness] [0-24]	4.10 (2.15)	4.18 (2.65)	4.14 (2.36)	0.79[b]	0.85	0.06
ISI [insomnia] [0-28]	2.85 (2.76)	2.82 (3.32)	2.84 (2.99)	0.52[b]	0.94	0.13
MEQ [chronotype] [16-86]	61.70 (9.11)	59.29 (6.27)	60.59 (7.92)	0.24[b]	0.87	0.23
PHQ-9 [depression] [0-27]	0.60 (1.14)	0.47 (0.80)	0.54 (0.99)	0.94[b]	0.94	0.02
GAD-7 [anxiety] [0-21]	0.70 (1.08)	0.47 (0.94)	0.59 (1.01)	0.47[b]	1.00	0.12
CCI total [subjective cognitive decline] [20-100]	27.00 (5.10)	30.59 (7.35)	28.65 (6.40)	0.05[b]	0.36	0.37

CCI memory [12-60]	18.05 (4.45)	20.00 (4.78)	18.95 (4.65)	0.16[b]	0.77	0.28
CCI executive functions [5-25]	4.95 (1.00)	5.65 (2.09)	5.27 (1.61)	0.56[b]	0.90	0.11
CCI language [3-15]	3.35 (0.49)	4.12 (1.45)	3.70 (1.10)	0.03[b]	0.44	0.38
m-ACE [global cognitive status] [0-30]	28.60 (1.43)	28.76 (1.15)	28.68 (1.29)	0.85[b]	0.88	0.04
SDMT [speed of processing] [0-110]	47.80 (5.60)	49.06 (8.21)	48.38 (7.18)	0.38[b]	1.00	0.17
TMT Part A (sec) [visuospatial attention]	28.85 (5.66)	27.59 (9.86)	28.27 (7.78)	0.63[a]	0.91	0.16
TMT Part A errors [visuospatial attention] [0-22]	0.20 (0.41)	0.35 (0.61)	0.27 (0.51)	0.48[b]	1.00	0.11
TMT Part B (sec) [visuospatial attention]	68.35 (23.35)	61.71(27.53)	65.30 (25.22)	0.43[a]	1.00	0.26
TMT Part B errors [visuospatial attention] [0-24]	0.15 (0.37)	0.71 (1.65)	0.41 (1.17)	0.48[b]	0.99	0.10
HVLT - Total Recall [verbal memory] [0-36]	25.00 (5.79)	27.71 (5.06)	26.24 (5.56)	0.12[b]	0.70	0.30
HVLT - Delayed Recall [verbal memory] [0-12]	8.30 (2.43)	9.88 (1.65)	9.03 (2.23)	0.04[b]	0.39	0.39
HVLT – Recognition [verbal memory] [0-12]	10.20 (1.47)	11.53 (0.70)	10.73 (1.31)	0.02[b]	0.58	0.44
ROCF – Copy [visuospatial abilities] [0-36]	33.58 (4.25)	34.32 (1.61)	33.92 (3.29)	0.74[b]	0.89	0.07
ROCF - Immediate Recall [visual memory] [0-36]	17.05 (7.68)	18.47 (5.98)	17.70 (6.90)	0.47[b]	1.00	0.14
ROCF - Delayed Recall [visual memory] [0-36]	16.77 (6.75)	18.68 (5.70)	17.65 (6.28)	0.48[b]	0.93	0.13
ROCF – Recognition [visual memory] [0-24]	19.40 (2.46)	20.24 (1.79)	19.78 (2.19)	0.23[b]	0.95	0.23
VST - Egocentric Navigation [0-7]	2.80 (1.74)	3.00 (1.32)	2.89 (1.54)	0.65[a]	0.90	0.09
VST - Allocentric Navigation	16.46 (10.01)	15.42 (7.03)	15.98 (8.67)	0.72[a]	0.95	0.12
VST - Heading Direction [spatial navigation] [0-7]	5.60 (1.39)	5.29 (1.49)	5.46 (1.43)	0.54[a]	0.92	0.12

Table 26. Demographic, psychological, sleep, sleepiness, chronotype and cognitive characteristics – SD vs MN sleep lab's protocols. Statistical tests and measures of effect sizes: a –independent sample t-test & Cohen's d), b –Mann-Whitney U Test & Rank-Biserial Correlation, c –Chi^2 & Cramer's V; Abbreviations: *CCI* - Cognitive Change Index; *PHQ-9* - Patient Health Questionnaire 9; *GAD-7* - General Anxiety Disorder 7; *PSQI* - Pittsburgh Sleep Quality Index; *ISI* - Insomnia Severity Index; *ESS* - Epworth Sleepiness Scale; *MEQ* – Morningness Eveningness Questionnaire; *m-ACE* - mini Addenbrooke's Cognitive Examination; *SDMT* - Symbol Digits Modalities Test; *TM* - Trail Making Test; *HVLT* - Hopkins Verbal Learning Test; *ROCF* - Rey Osterrieth Complex Figure; *VST* – Virtual Supermarket Task; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj).

6.6.3. Digit Span task and Raven Progressive Matrices

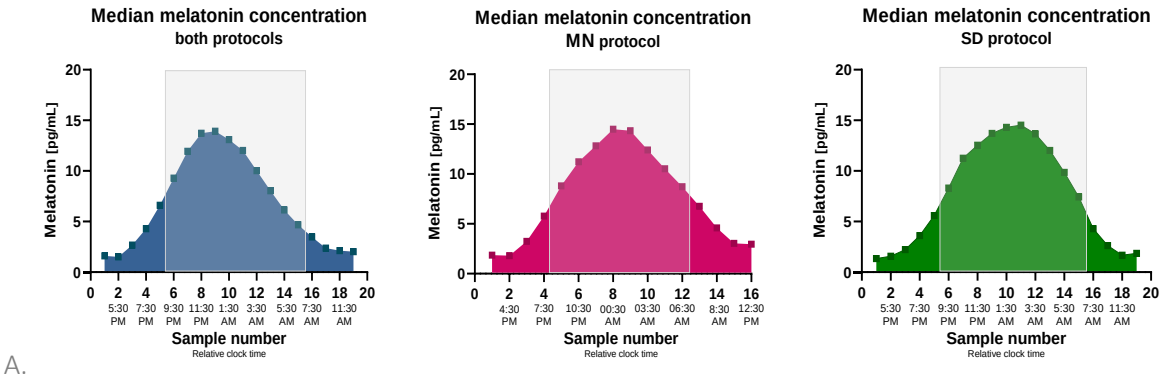
To further evaluate the cognitive profile of the APOE-ε4 allele carriers and non-carriers, two voluntary neuropsychological tests were assessed following the recovery night. Neither, the measure of fluid intelligence assessed by the Raven Progressive Matrices, nor verbal short-term and working memory evaluated by the Digits Span task yielded notable differences between the two APOE risk groups (Table 27). The Cohen's *d* effect sizes ranged from none to small for all outcome measures.

Outcome measures [range]	APOE-ε4+ (N=18) (M (SD)	APOE-ε4- (N=14) (M (SD)	Total (N=32) (M (SD)	p	p-adj	Effect size
Raven Total Set A [0-12]	11.17 (1.15)	11.14 (1.03)	11.16 (1.08)	0.95[a]	0.95	0.02
Raven Total Set B [0-12]	10.67 (2.00)	11.50 (0.52)	11.03 (1.58)	0.39[b]	1.00	0.17
Raven Total Set C [0-12]	9.94 (1.92)	10.21 (1.19)	10.06 (1.06)	0.65[a]	1.00	0.16
Raven Total Set D [0-12]	9.89 (1.71)	10.86 (0.77)	10.31 (1.45)	0.10[b]	0.9	0.33
Raven Total Set E [0-12]	7.06 (2.10)	7.50 (1.07)	7.25 (2.58)	0.64[a]	1.00	0.17
Raven Total Score [0-60]	48.72 (8.28)	51.21 (3.58)	49.81 (6.67)	0.76[b]	1.00	0.07
Digits Span forward [0-8]	5.89 (1.23)	6.00 (1.46)	5.94 (1.37)	0.82[a]	0.92	0.08
Digits Span backwards [0-7]	4.61 (1.24)	4.25 (1.44)	4.44 (1.33)	0.44[a]	1.00	0.27
Digits Span Total Score [0-15]	10.50 (1.33)	10.25 (2.72)	10.38 (2.49)	0.78[a]	1.00	0.10

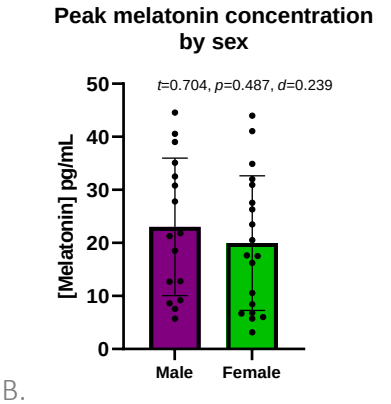
Table 27. Raven Progressive Matrices and Digits Span task scores comparison between APOE-ε4 allele carriers and controls (ε3/ε3). Statistical tests and measures of effect sizes: a –independent sample t-test & Cohen's *d*, b –Mann-Whitney U Test & Rank-Biserial Correlation. Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).

6.6.4. Melatonin

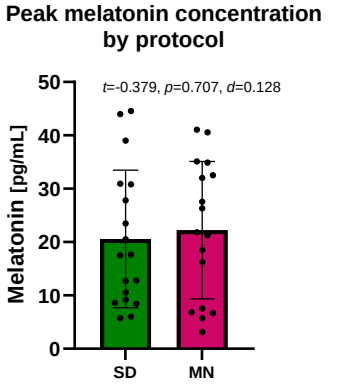
Melatonin concentration followed the expected pattern, i.e., concentration was raising gradually starting in the late afternoon with a peak concentration between midnight and 3 AM which was followed by a gradual decrease throughout the second part of the night with very low concentrations in the morning hours for both protocols (Figure 34A). ANCOVA analysis involving peak melatonin concentration (i.e., an average of three highest values) controlled for age, sex, APOE-ε4 status and protocol revealed only a trend towards significance for age (F=3.467, p=0.07, η^2=0.10). There were no differences in peak melatonin concentration between males and females (t=0.70, p=0.49, d=0.24) (Figure 34B), between Sleep deprivation and Multinap protocols (t=0.38, p=0.71, d=0.13) (Figure 34C) or APOE-ε4 allele carriers and controls (ε3/ε3) (U=0.32, p=0.70, r_{rb}=0.11) (Figure 34D). No significant interaction was found between peak melatonin concentration, APOE status and protocol (F=0.05, p=0.83, η^2=0.00) (Figure 34E). All individual melatonin profiles including basic demographic information and the protocol the participant was enrolled in can be found in Appendix 13 – *Melatonin Individual plots.*



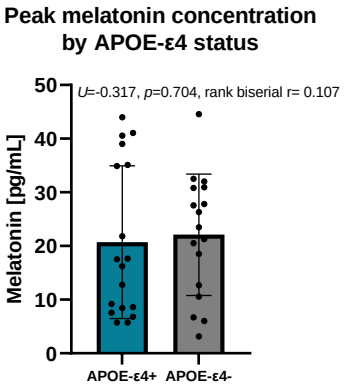
A.



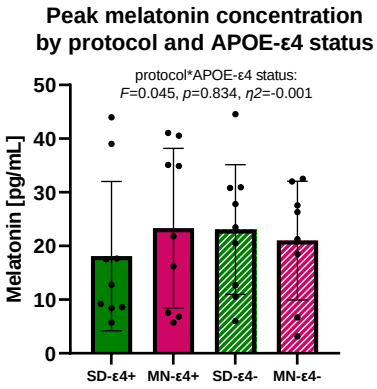
B.



C.



D.



E.

Figure 34. Melatonin concentrations. The top panel (A) shows median values of raw melatonin concentrations with corresponding times of sampling according to each protocol. Note that the timing before the protocols was slightly different because of the 80-minute long naps scheduled during the MN protocol. Data on the graphs were smoothed (2nd order smoothing (4 neighbours)) for demonstrative purposes. Figures in the two remaining panels show peak melatonin concentration between males and females (B), SD and MN protocols (C), APOE-ε4 carriers and controls (ε3/ε3) (D) and APOE-ε4 status and protocols interaction (E).

6.6.5. Cognitive assessment

Two Fit Linear Mixed-Effects Models (lmer) with participants (i.e., (1|id) as random effects were used to analyse the cognitive outcome measures:

- *Model 1 was used to investigate the effect of the protocol and its interactions with a time of the day (i.e. session number) controlled for confounding factors:*

lmer(variable of interest ~ age + sex + protocol + session number + years of education + hours of sleep baseline + protocol*session number + (1|id)

- *Model 2 was performed to investigate the effect of APOE-ε4 status and its interactions with the protocol and time of the day controlled for confounding factors:*

lmer(variable of interest ~ age + sex + APOE-ε4 status + protocol + session number + years of education + hours of sleep baseline + protocol*session number + protocol* APOE-ε4 status + protocol*session number* APOE-ε4 status + (1|id)

The tables with detailed statistics for each plotted variable are organized in descending order of the effect size and can be found in Appendix 14 - *Statistics - Model 1* and Appendix 15 - *Statistics Model 2*.

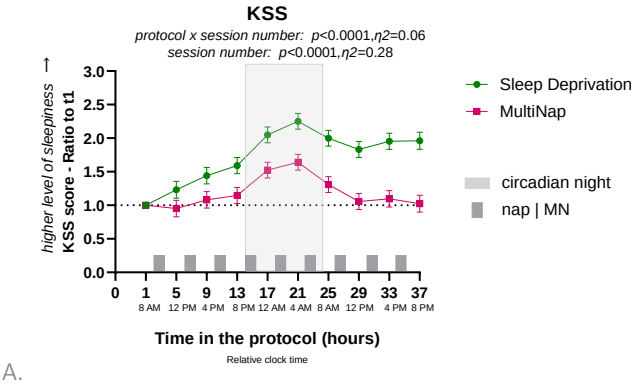
6.6.5.A. To what extent are objectively and subjectively measured vigilance, working memory, episodic memory performance and subjective mental effort affected by low and high sleep pressure conditions and time of day (i.e. circadian phase) in healthy elderly men and women?

To evaluate if sleep pressure modulation was successful, performance between SD and MN groups was compared using well-validated measures that are commonly used in experimental sleep and circadian protocols (see Introduction - Appendix 1), i.e., Karolinska Sleepiness Scale (KSS), Psychomotor Vigilance Task (PVT), n-back and mental effort (PVT and the cognitive assessment overall). Additionally, a novel Episodic long-term memory task was analysed to investigate the effect of sleep restriction *and time of the day* on episodic memory performance. Those cognitive outcomes were expressed as a ratio to the first cognitive assessment following the Baseline Night (t1), i.e., each assessment (t2-t10) was divided by t1 performance.

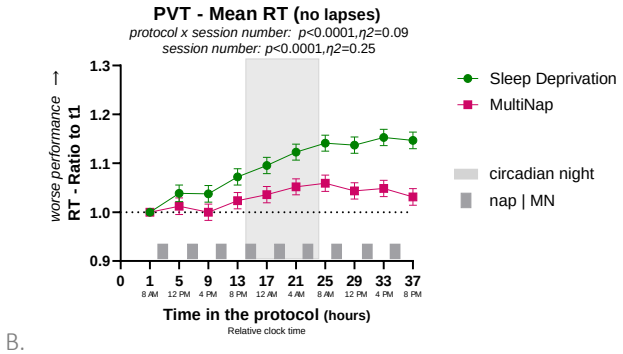
Subjective sleepiness (KSS) and objective measure of vigilance (PVT)

Time spent awake was associated with a gradual increase in subjective sleepiness (KSS) (p<0.0001, η^2=0.06) (Figure 35A) and an increase in the PVT-assessed averaged RT (p<0.0001, η^2=0.09) (Figure 35B), median RT (p<0.0001, η^2=0.10), 10% fastest RT (p<0.0004, η^2=0.05), decrease in slowest 10% RT

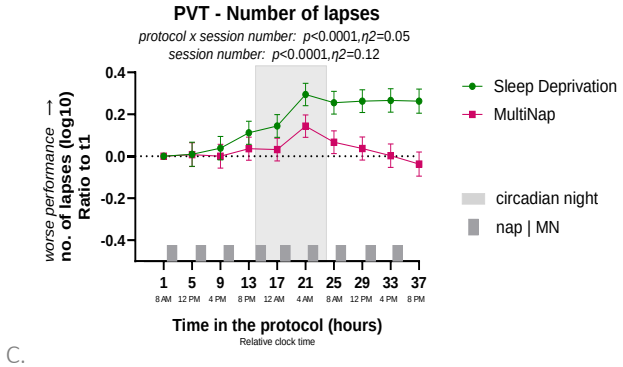
(p=0.05, η^2=0.02) and increased number of lapses (p=0.002, η^2=0.05) (Figure 35C) in SD compared to MN. KSS and PVT-number of lapses followed a circadian pattern with the highest scores reported during the circadian night (KSS: p<0.0001, η^2=0.28; PVT-number of lapses: p<0.001, η^2=0.12) (Figure 35A and C).



A.



B.



C.

Figure 35. Subjective sleepiness and objective vigilance throughout 40h of SD and MN protocols. **A.** KSS, **B.** PVT – averaged reaction time (without lapses) **C.** PVT – number of lapses (RT≥500ms). Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol Data points are expressed as least-square means values ±SE and are a ratio to timepoint t1 which was considered as the baseline value.

Working memory (n-back)

RT in the one-back condition was significantly worse in SD compared to the MN condition (one-back RT p=0.05, η^2=0.02) (Figure 36A) and followed a circadian pattern with the slowest RT during the circadian night (p<0.0001, η^2=0.22). Two-back accuracy showed worse performance for both protocols during the circadian night that was followed by improvement during the wake maintenance zone, i.e., in the evening hours (p=0.04, η^2=0.08) (Figure 36D). Further, two-back accuracy showed a main effect of the protocol (p=0.03, η^2=0.10) with a significantly worse performance in SD-randomized participants that was the most prominent in the second part of the protocol (p<0.0001, η^2=0.11) (Figure 36D). No significant main effects were found in two-back RT (Figure 36B). Please, note that accuracy scores were raised to high power to normalize the residuals resulting from the applied lmer (Figures 36C and 36D).

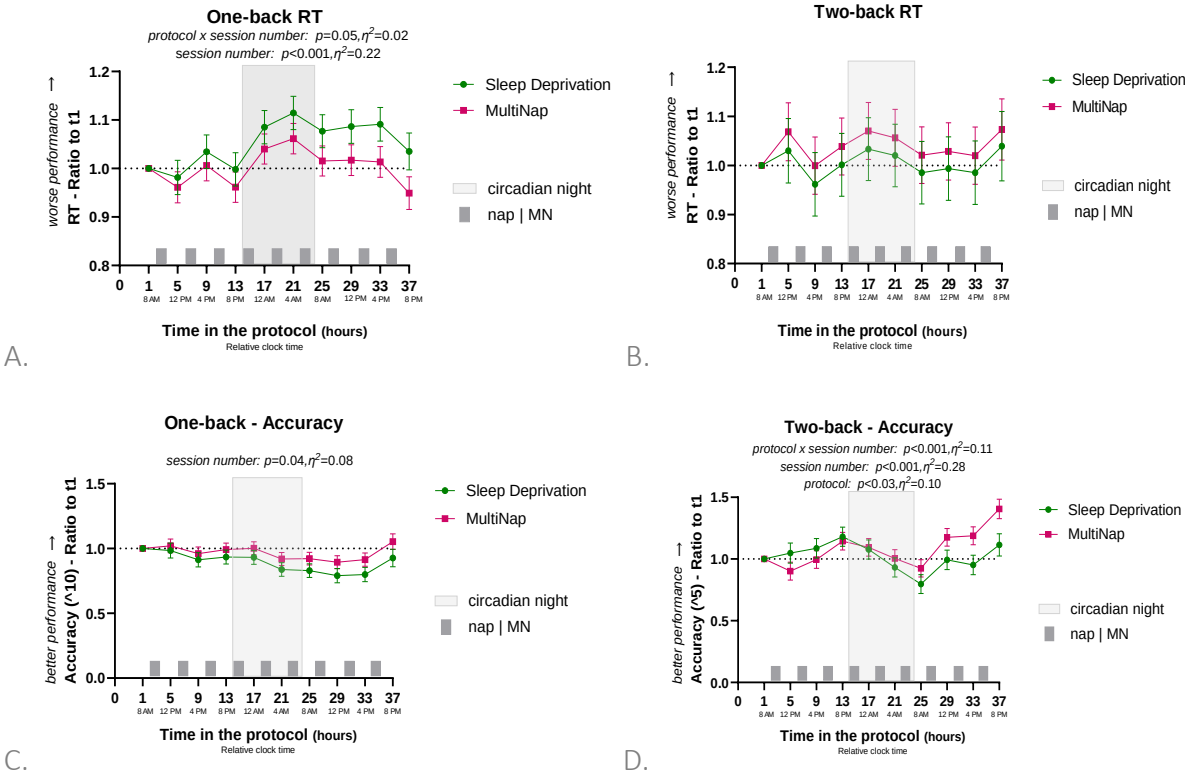


Figure 36. Working memory performance was assessed by an n-back test throughout 40h of SD and MN protocols. A. RT for the One-back condition, B. RT for the Two-back condition, C. Accuracy for One-back, and D. Accuracy for Two-back. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Mental effort

Subjective cognitive demand relative to baseline values across all cognitive tests was higher in SD relative to MN, with the experimental condition effects becoming gradually stronger with time spent in the study and particularly on the second day of the lab session (p=0.001, η^2=0.04) (Figure 37A). The highest cognitive demand was indicated during the second day in the SD protocol (p=0.002, η^2=0.09).

Mental effort for PVT was likewise higher in SD compared to MN and while it was relatively constant in MN, in SD protocol, the task required significantly more cognitive effort to complete with each coming cognitive session (p=0.04, η^2=0.02) (Figure 37B).

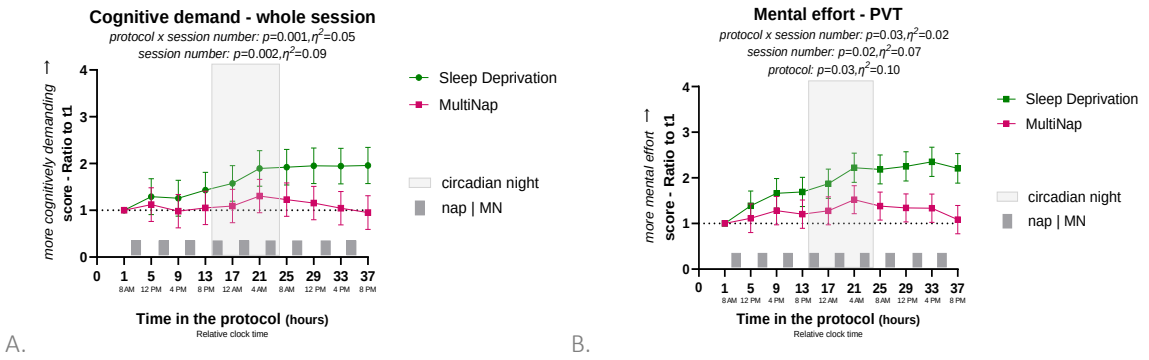


Figure 37. Mental effort was assessed throughout 40h of SD and MN protocols. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. A. Subjective cognitive demand – whole session, B. Mental effort – PVT task. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Episodic memory

Overall, neither time to complete the whole task (i.e., Recognition and Source Memory), the reaction time of Hits, Source Memory Hits, nor the number of Source memory hits demonstrated the time of the day effect (i.e. session number)_or protocol effect (Figure 38A, B, D and E). There was only a marginally significant *protocol* and *session number effect* for a reaction time of Correct Rejections (p=0.08, η^2=0.08), where participants in the MN protocol had lower RT during the first day of the protocol compared to controls (ε3/ε3) (Figure 38C). The RT in the MN group was decreasing with each assessment over the second day of the lab session. Notably, repeated exposure effect (practice) can be seen in, for instance, *time to complete the whole task* where reaction times were getting faster relative to the baseline during both protocols (Figure 38A).

Importantly, the Recognition task was characterized by a prominent ceiling effect. In MN, the Median Hits across ten-session ranged from 93% to 100%, while the mode was 100 for all sessions besides sessions 3, 4, and 7 where it was 93. In SD, the Median Hit ranged from 93% to 100%, the Mode Hits were 100% besides session number 6. Due to a very small range of outcome values, the distributions residuals from the mixed models were skewed and could not be normalized, hence the results of the models did not explain the trends in the examined database. The residuals of reaction times were normally distributed but are not informative outcomes for the task (see *Discussion – Limitations related to cognitive tasks*).

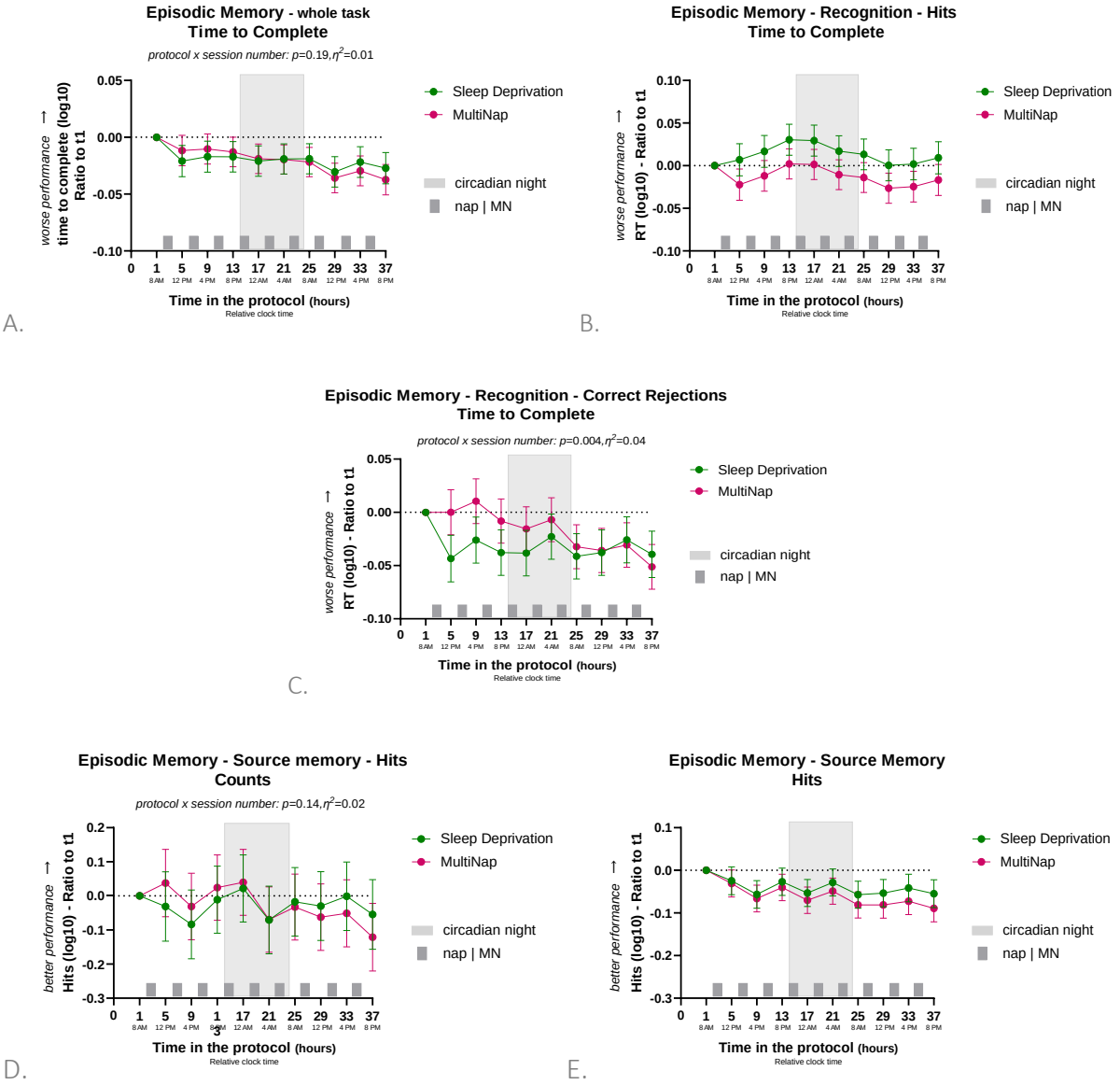


Figure 38. Episodic memory performance was assessed by Episodic Memory task throughout 40h of SD and MN protocols. **A.** time to complete – whole task (i.e., Encoding and Recognition sub-tasks), **B.** RT - Recognition - Hits, **C.** RT – Recognition – Correct Rejections, **D.** Number of Hits – Source Memory, **E.** RT – Source Memory – Hits. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

6.6.5.B. What is the effect of sleep pressure and time of the day on allocentric and egocentric navigation?

Allocentric spatial navigation (SHQ-Wayfinding levels)

Among allocentric navigation outcome measures, only time to complete-easy levels revealed time of a day and protocol effects (p=0.04, η^2=0.02) (Figure 39C). The interaction demonstrates that participants randomized into the MN condition were faster in completing the task, especially during the second day of the experimental protocol. The self-reported mental effort showed marginally higher

demand to complete wayfinding easy and hard levels in MN compared to SD protocol (*easy levels:* p=0.06, η^2=0.08; *hard levels:* p=0.17, η^2=0.05) (Figure 39E and F). Further, more advanced age was positively associated with a higher rating of mental effort, for both easy (p=0.04, η^2=0.16) and hard levels (p=0.05, η^2=0.15). There was also an independent effect of sex for mental effort at easy levels (p=0.04, η^2=0.16) and a trend towards significance for hard levels (p=0.08, η^2=0.12), where females inclined to report higher mental effort required to complete easy ($p \leq 0.001$, d=0.68, male: 4.55±2.43; female: 6.24±2.51) and hard levels ($p \leq 0.001$, d=0.65, male: 5.46±2.24; female: 6.95±2.33). Notably, longer competition time was associated with long-distance travelled, i.e., poorer performance for easy (*rho*=0.94, $p \leq 0.001$) and hard levels (*rho*=0.95, $p \leq 0.001$).

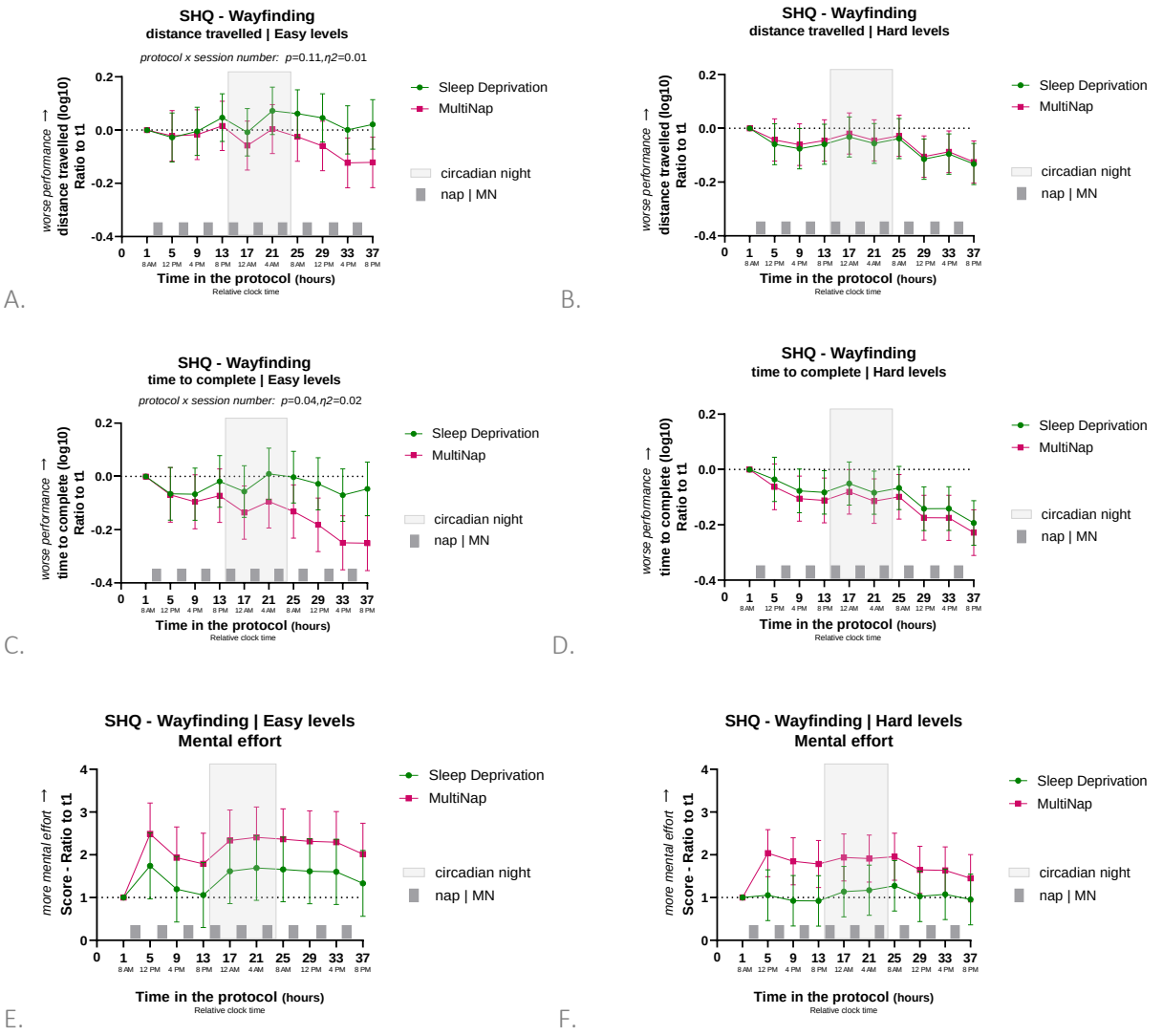
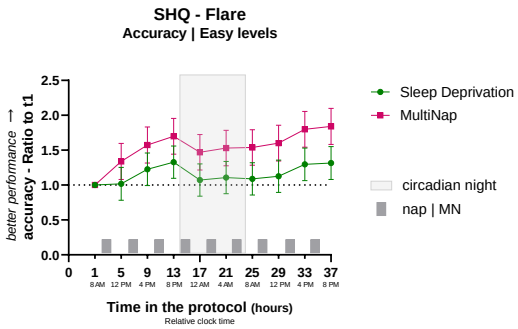


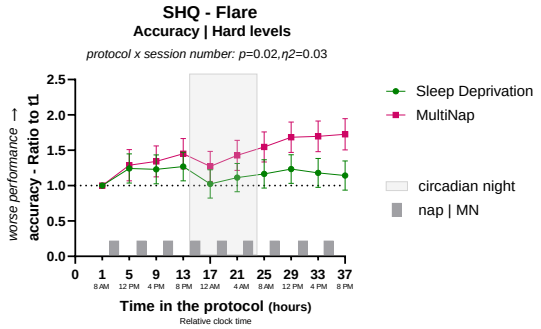
Figure 39. Allocentric navigation performance was assessed by the Sea Hero Quest task (wayfinding levels) throughout 40h of SD and MN protocols. A. Wayfinding – distance travelled – Easy levels, B. Wayfinding – distance travelled – Hard levels, C. Wayfinding – time to complete – Easy levels, D. Wayfinding – time to complete – Hard levels, E. Wayfinding – subjective Mental effort – Easy levels, F. Wayfinding – subjective Mental effort – Hard levels. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Egocentric spatial navigation (SHQ-Flare levels)

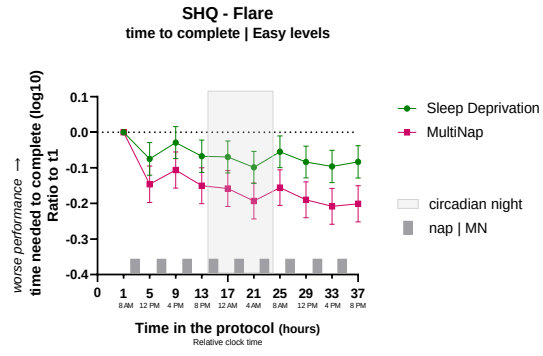
The accuracy level for the easy levels has not yielded significance for any of the main effects and did not show the time-of-a-day or protocol effect (Figure 40A). However, for the MN protocol, accuracy for hard levels showed significant and steady raising improvement throughout the second day of the protocol (p=0.02, η^2=0.03) which is more likely an indicator of learning effect than circadian modulation (Figure 40B). Further, participants in the MN protocol tend to take less time to complete easy flare levels with the best performance during afternoon and evening cognitive sessions over the second day of the protocol (p=0.08, η^2=0.01) (Figure 40C). For competition time-hard levels, however, participants in the SD protocol performed the task faster yet the difference was the most prominent during the first day of the protocol ($p \leq 0.001$, η^2=0.06) (Figure 40D). Additionally, protocol effect was demonstrated also in a mental effort - easy flare levels with individuals in SD protocol indicating more effort invested (p=0.05, η^2=0.10) (Figure 40E). Moreover, elderly participants tend to indicate higher mental effort demand for easy (p=0.06, η^2=0.14) and hard levels (p=0.05, η^2=0.15). Importantly, no association was found between the time needed to complete (sec) and flare accuracy neither for easy (rho=0.02, p=0.75) nor hard levels (rho=-0.05, p=0.41).



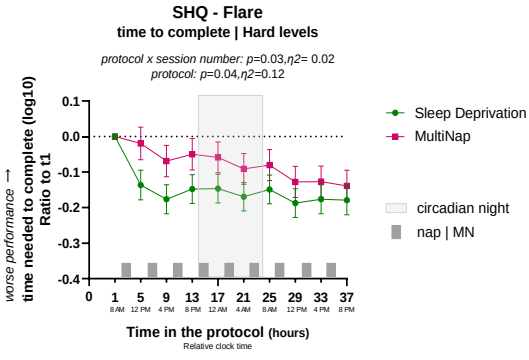
A.



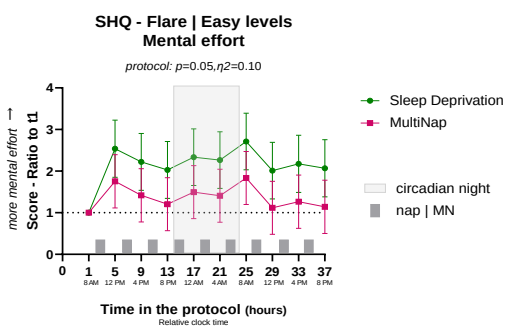
B.



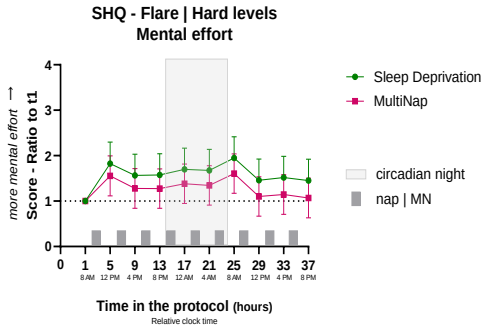
C.



D.



E.



F.

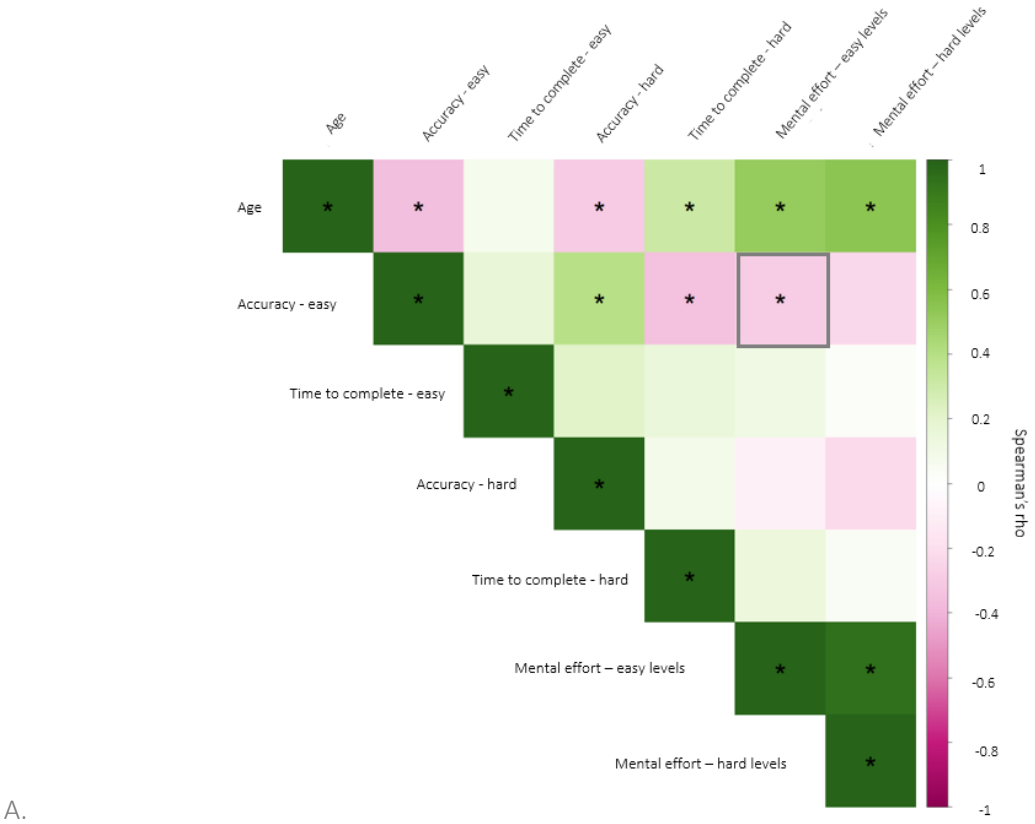
Figure 40. Egocentric navigation performance was assessed by the Sea Hero Quest task (flare levels) throughout 40h of SD and MN protocols. A. Flare – accuracy – Easy levels, B. Flare – accuracy – Hard levels, C. Flare – time to complete – Easy levels, D. Flare – time to complete – Hard levels, E. Flare – subjective Mental effort – Easy levels, F. Flare – subjective Mental effort – Hard level. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

SHQ – Easy vs Hard levels

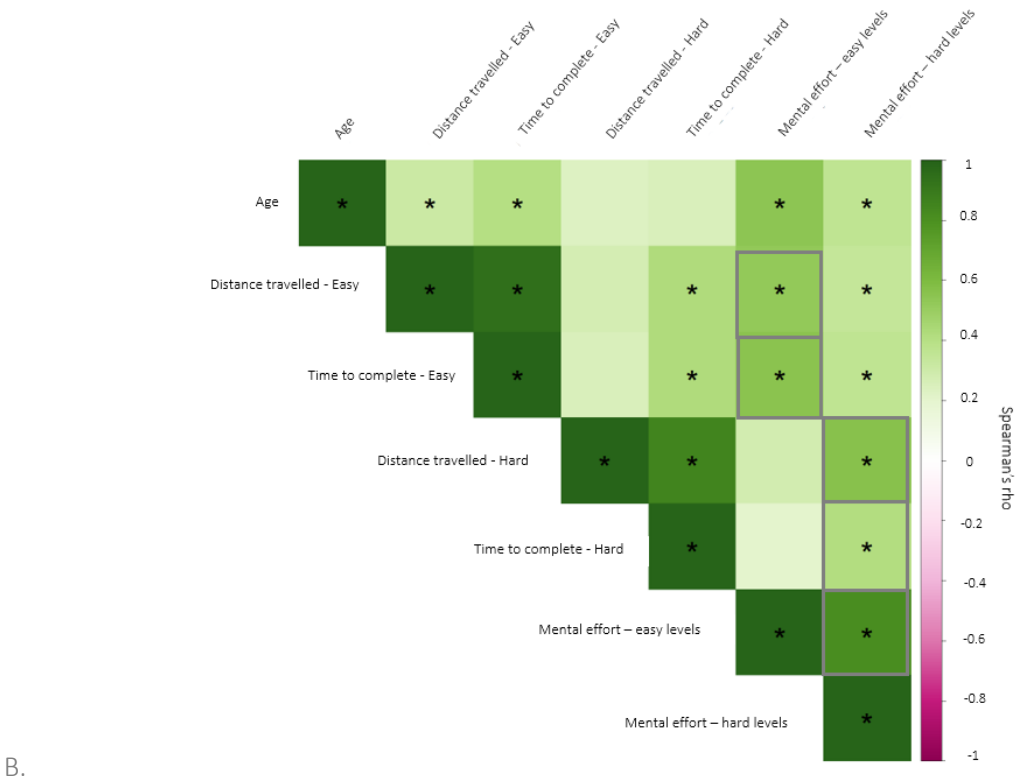
There was a significant difference between easy and hard levels in the wayfinding task where the overall distance travelled was longer (U=22307, p<0.001, r_{rb}=0.50) and took more to complete (U=26159, p=0.001, r_{rb}=0.41) for hard levels (averaged distance for easy levels($M\pm SD$): 1.55±0.93, averaged distance for hard levels($M\pm SD$): 2.44±1.18; the average time to complete for easy levels($M\pm SD$): 85.38±65; the average time to complete for hard levels($M\pm SD$): 127.44±81.97). There was also a notable difference between easy and hard flare levels for the time needed to complete (U=18020, p<0.001, r_{rb}=0.56) (averaged time to complete for easy levels($M\pm SD$): 19.88±7.74; time to complete for hard levels($M\pm SD$): 28.16±18.47) but not for flare accuracy (X^2=0.55, p=0.76). Raw values from both protocols, from all sessions combined, were used for the foregoing calculations.

SHQ and Mental effort scale

Spearman correlation coefficient involving SHQ tasks and mental effort scale, where the participants were asked how much mental effort was required to complete easy and hard levels for each flare and wayfinding tasks were run. Note that the p-value was kept at a level ≤0.15 to explore potential trends. For, egocentric navigation (flares), only the association between accuracy Flare – easy levels and mental effort – Flare easy levels yielded significance (p≤0.0001) (Figure 41A). For allocentric navigation assessed by SHQ wayfinding levels, the mental effort scale was much closer associated with objective assessment (Figure 41B). As listed in Table 28, a higher mental effort for easy and hard levels, for both distance travelled and time to complete was positively associated with poorer performance. The rho values oscillated between 0.42 to 0.57 indicating low to moderate correlations. Furthermore, more advanced age was correlated with self-reported higher mental effort demand to complete both easy and hard wayfinding levels.



A.



B.

Figure 41. Adjacency matrix showing associations spatial navigation assessed by Sea Hero Quest and mental effort scale related to the task. The upper panel (A.) shows egocentric navigation (SHQ-Flare) and the lower one (B.) allocentric component (SHQ-Wayfinding) variables of interest (SHQ-wayfinding). Stars indicate *p*-values at ≤0.15. The colour scale indicates the strength and direction of the correlation. The further Spearman's ρ is from zero, the stronger the association between the variables. The sign of ρ indicates the direction of the relationship. Significant correlations that survived Benjamini-Hochberg's correction are highlighted in grey.

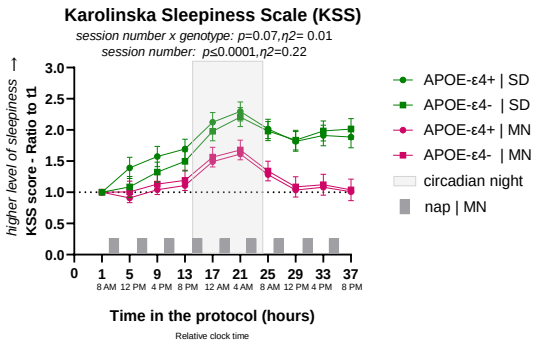
Variables	Mental effort – Easy levels			Mental effort – Hard levels		
	Spearman's rho	*p*	*p-adjusted*	*Spearman's rho*	*p*	*p-adjusted*
age	0.55	0.003	0.01	0.36	0.06	0.06
Distance travelled - Easy	0.52	0.005	0.01	_	_	_
Time to complete - Easy	0.55	0.003	0.01	_	_	_
Distance travelled - Hard	_	_	_	0.57	0.002	0.01
Time to complete - Hard	_	_	_	0.42	0.03	0.05

Table 28. Significant correlations for Mental effort (easy and hard levels) and SHQ wayfinding outcome measures (shown in Figure 41 A and B). Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj).

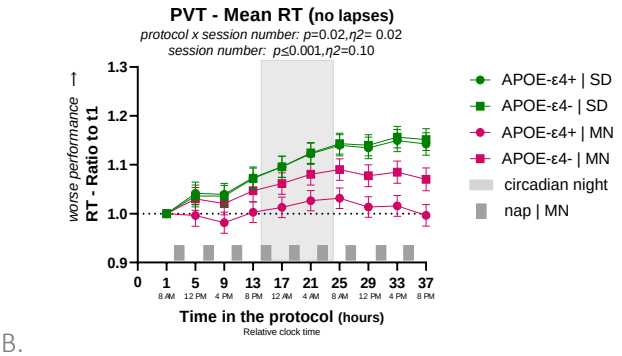
6.6.5.C. Does the genetic risk of AD (ε4 allele carriership) modulate the effect of sleep loss and time of the day on objectively and subjectively measured vigilance, working memory, episodic memory and subjective mental effort?

Subjective sleepiness (KSS) and objective measure of vigilance (PVT)

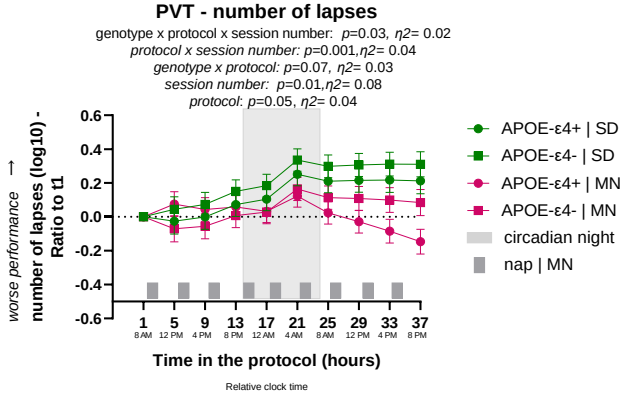
Subjective sleepiness was modulated by the time of day with at-risk participants in the SD protocol reporting a marginally higher level of sleepiness during the first day of the protocol (p=0.07, η^2=0.01) compared to controls (ε3/ε3) (Figure 42A). For PVT, the number of lapses was modulated by the time of the day with the highest number of lapses during the circadian night followed by lapses reduction during the second day of the protocol that maintained a similar level in all groups besides at-risk MN participants whose laps' number kept decreasing with each assessment (t7-t10) (p=0.03, η^2=0.02) (Figure 42C). The largest effect sizes were obtained by age for the number of lapses (η^2=0.21), years of education for mean RT (η^2=0.19) and time-of-a-day (session number) for KSS (η^2=0.22). The main effects involving APOE-ε4 status oscillated between negligible to low.



A.



B.

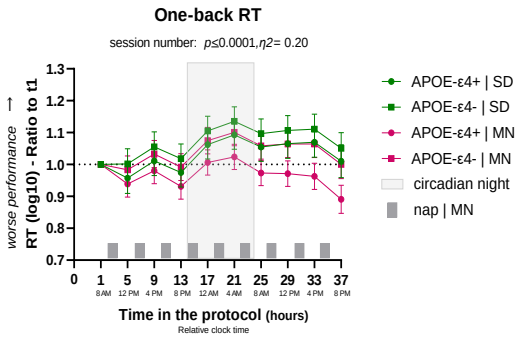


C.

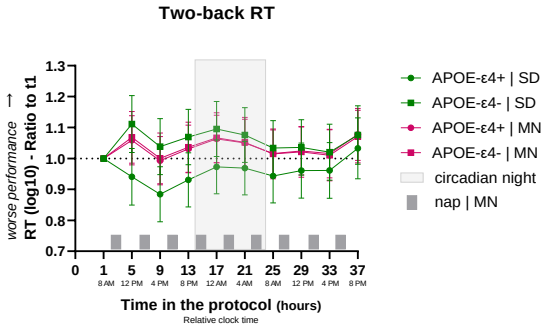
Figure 42. Subjective and objective measures of vigilance throughout 40h of sleep modulation protocols. The graphs show a comparison between APOE-ε4 allele carriers and controls (ε3/ε3) in SD and MN protocols. A. KSS, B. PVT – averaged reaction time (without lapses) C. PVT – number of lapses (RT≥500ms). Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Working memory (n-back)

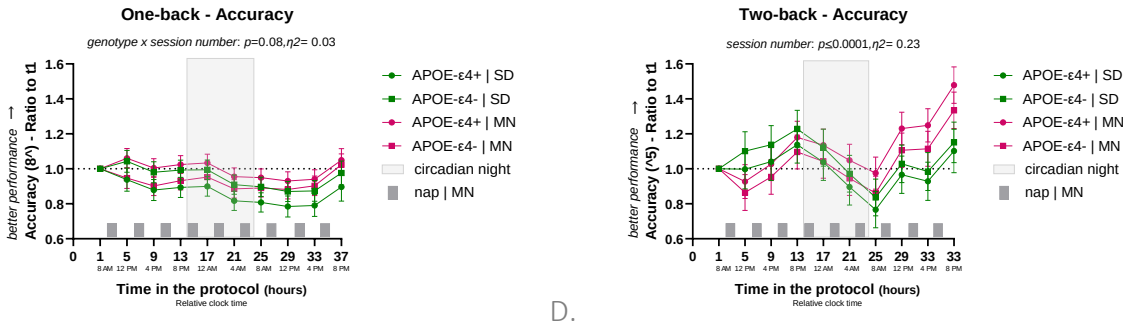
No genotype differences were found across n-back outcome measures besides a trend toward significance for one-back accuracy, where at-risk participants in the SD condition performed marginally worse during the second day of the protocol (p=0.08, η^2=0.03) (Figure 43A). The performance improved, however during the last cognitive assessment (t10, wakefulness-promoting zone). All of the effect sizes concerning APOE-ε4status were none or small.



A.



B.

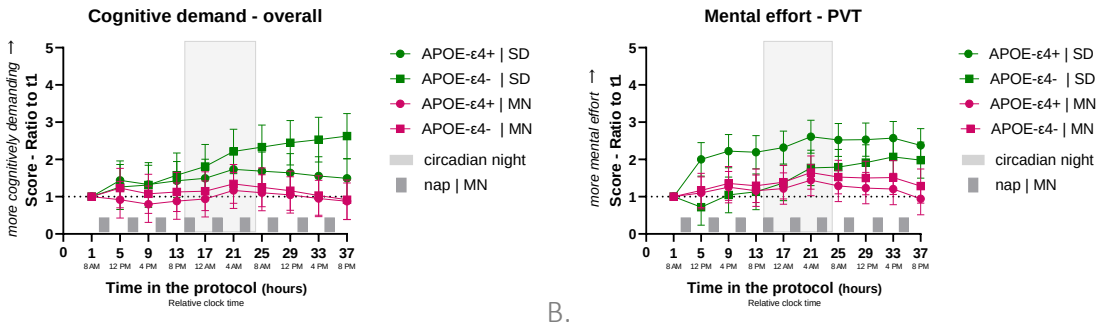


C. D.

Figure 43. Working memory performance was assessed by an n-back test throughout 40h of SD and MN protocols. The graphs show *a* comparison between APOE-ε4 allele carriers and controls (ε3/ε3) in both protocols. A. RT for the One-back condition, B. RT for the Two-back condition, C. Accuracy for One-back, and D. Accuracy for Two-back. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Mental effort

The negative effect of sleep restriction on the level of cognitive demand was significantly modulated by the time of the day and APOE-ε4 carriership. The controls (ε3/ε3) in the SD protocol showed a higher level of cognitive demand required to complete the cognitive assessments with the scores raising noticeably from the early morning hours of the second day of the protocol (p=0.001, η^2 =0.04) (Figure 44A). Further, at-risk participants indicated marginally higher mental effort demand to complete the PVT task compared to the controls (ε3/ε3) in both SD and MN protocols (p=0.06, η^2=0.10). The effect sizes returned from the multivariate models ranged from none to medium with terms involving genotyping being medium for PVT mental effort and small for cognitive demand.



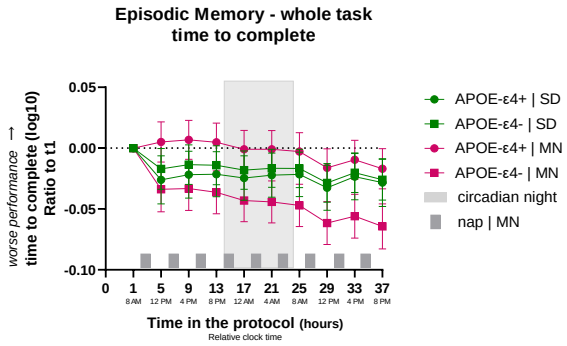
A. B.

Figure 44. Mental effort was assessed using the VAS scale throughout 40h of protocols. The graphs show *a* comparison between APOE-ε4 allele carriers and controls (ε3/ε3) in SD and MN protocols. A. Subjective cognitive demand – whole session, B. Mental effort – PVT task. Data points are expressed as least-square means values. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

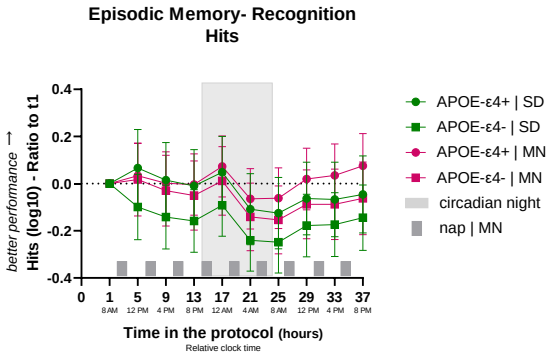
Episodic memory

Overall, the performance on the Episodic memory task has not been shown to be under either strong homeostatic regulation or the time of the day or modulated by APOE-ε4 status (Figure 45 A-C). Years of education represented the significant predictor with large effect sizes for RT of Recognition Hits (p=0.02, η^2=0.22) and number of Hits for Source Memory (p=0.05, η^2=0.16). Source Memory – Hits RT was marginally better in at-risk participants enrolled in the MN protocol compared to controls (ε3/ε3) in the MN and for controls (ε3/ε3) in the SD compared to ε4 carriers in the SD (p=0.06, η^2=0.07) (Figure 45E).

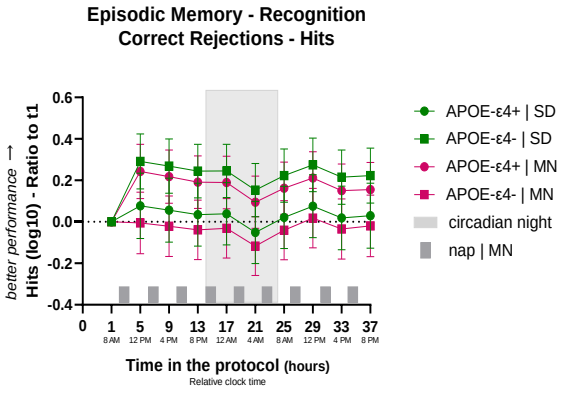
The Recognition task was characterized by a prominent ceiling effect. In both the MN and SD protocols the Median and the Mode of the Hits measured across ten sessions ranged from 93% to 100% (for both ε4+ and ε4-). Due to a very small range of scores, the distributions of mixed models were not normally distributed and could not be normalized, hence the results of the models did not explain the trends in the examined database. The residuals of reaction times were normally distributed but are not informative outcomes for the task (see limitation section in the *Discussion*).



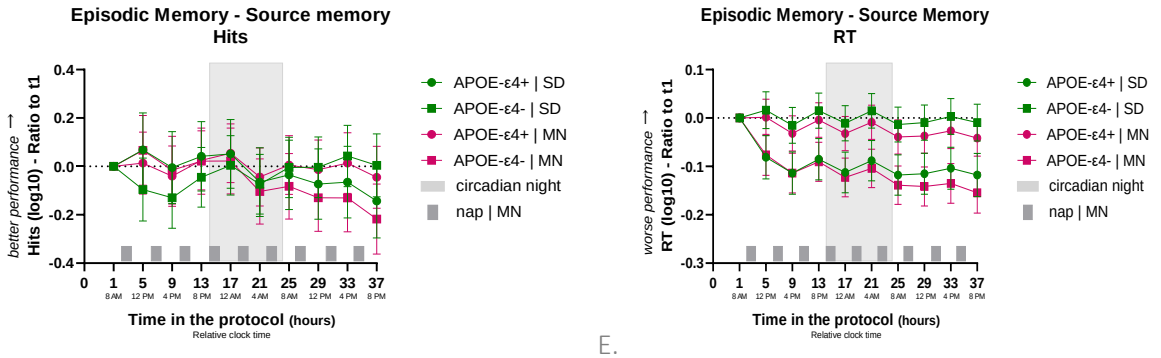
A.



B.



C.



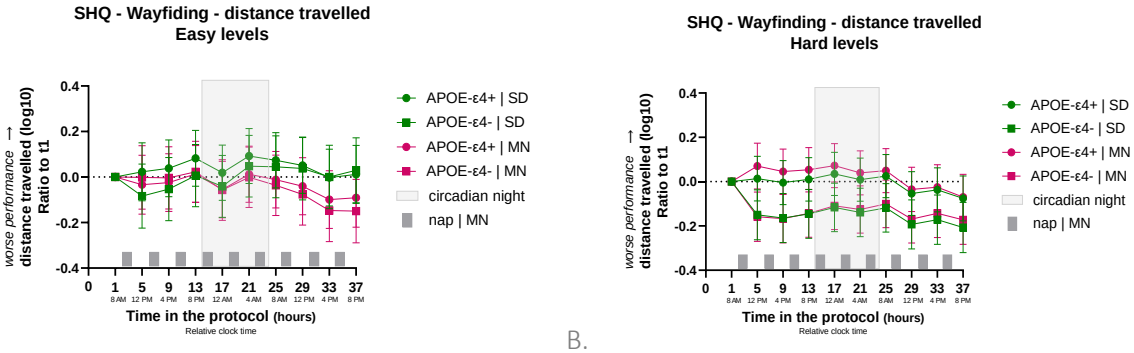
D. E.

Figure 45. Episodic memory performance was assessed by Episodic Memory task throughout 40h of SD and MN protocols. The graphs show a comparison between APOE-ε4 allele carriers and controls (ε3/ε3) in both protocols. **A.** time to complete – the whole task (i.e., Encoding and Recognition sub-tasks), **B.** RT - Recognition - Hits, **C.** RT – Recognition – Correct Rejections, **D.** Number of Hits – Source Memory, **E.** RT – Source Memory – Hits. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

6.6.5.D. Does the genetic risk of AD (ε4 allele carriership) modulate the effect of sleep loss and time of the day on spatial navigation?

Allocentric spatial navigation (SHQ-Wayfinding levels)

Neither an effect of sleep restriction nor a modulation by the time of the day nor an APOE-ε4 status effect was found in SHQ performance or related mental effort rating (Figure 46 A-F). Only the time to complete the wayfinding task at easy levels was marginally lower during the second day of the protocol for participants randomized into MN, especially at-risk individuals (p=0.001, η^2=0.01), however, it can be attributed more to the learning effect than the time of the day effect. Note that most of the reported effect sizes are negligible.



A. B.

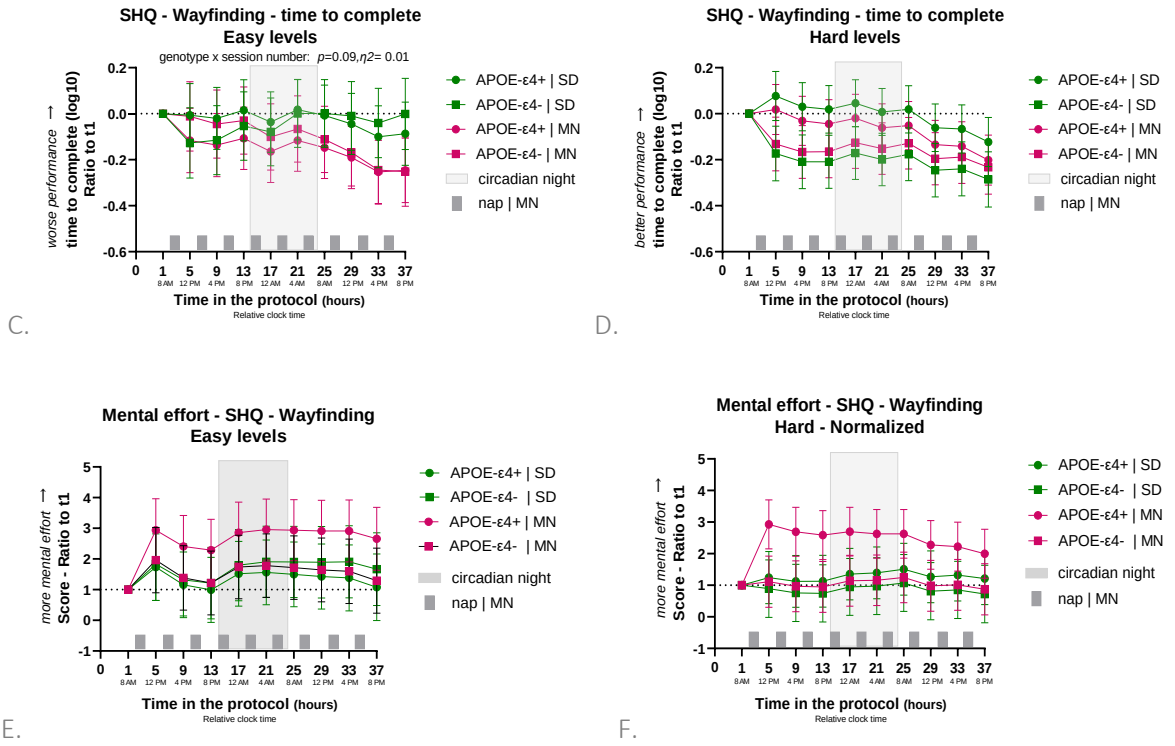


Figure 46. Allocentric navigation performance was assessed by the Sea Hero Quest task (wayfinding levels) throughout 40h of SD and MN protocols. The graphs show a comparison between APOE-ε4 allele carriers and controls (ε3/ε3) in SD and MN protocols. A. Wayfinding – distance travelled – Easy levels, B. Wayfinding – distance travelled – Hard levels, C. Wayfinding – time to complete – Easy levels, D. Wayfinding – time to complete – Hard levels, E. Wayfinding – subjective Mental effort – Easy levels, F. Wayfinding – subjective Mental effort – Hard levels. Data points are expressed as least-square means values. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

Egocentric spatial navigation (SHQ-Flare levels)

The flare SHQ sub-task showed the protocol differences in the time needed to complete easy flare levels. While participants in the low sleep-pressure condition (i.e. MN) required less time to complete the task throughout the protocol (p=0.02, η^2=0.02), the beneficial effect of lower sleep pressure was only marginally noticeable in at-risk individuals (p=0.08, η^2=0.01) (Figure 47C). Moreover, at-risk participants in the MN condition had marginally higher flare accuracy at hard levels (p=0.07, η^2=0.08) compared to SD counterparts and controls (ε3/ε3) in both protocols (Figure 47B). The negative effect of sleep restriction on mental effort rating for hard flare levels was marginally higher in the control group (p=0.09, η^2=0.00) yet the effect size is negligible (Figure 47F). No genotyping effect was found for easy flare accuracy, time to complete-hard levels or mental effort- easy levels (Figure 47A, 47D, 47E). Note that age reached the biggest effect size for flare accuracy (easy levels: η^2= 0.11, hard levels: η^2=0.13) and time to complete (hard levels: η^2=0.15).

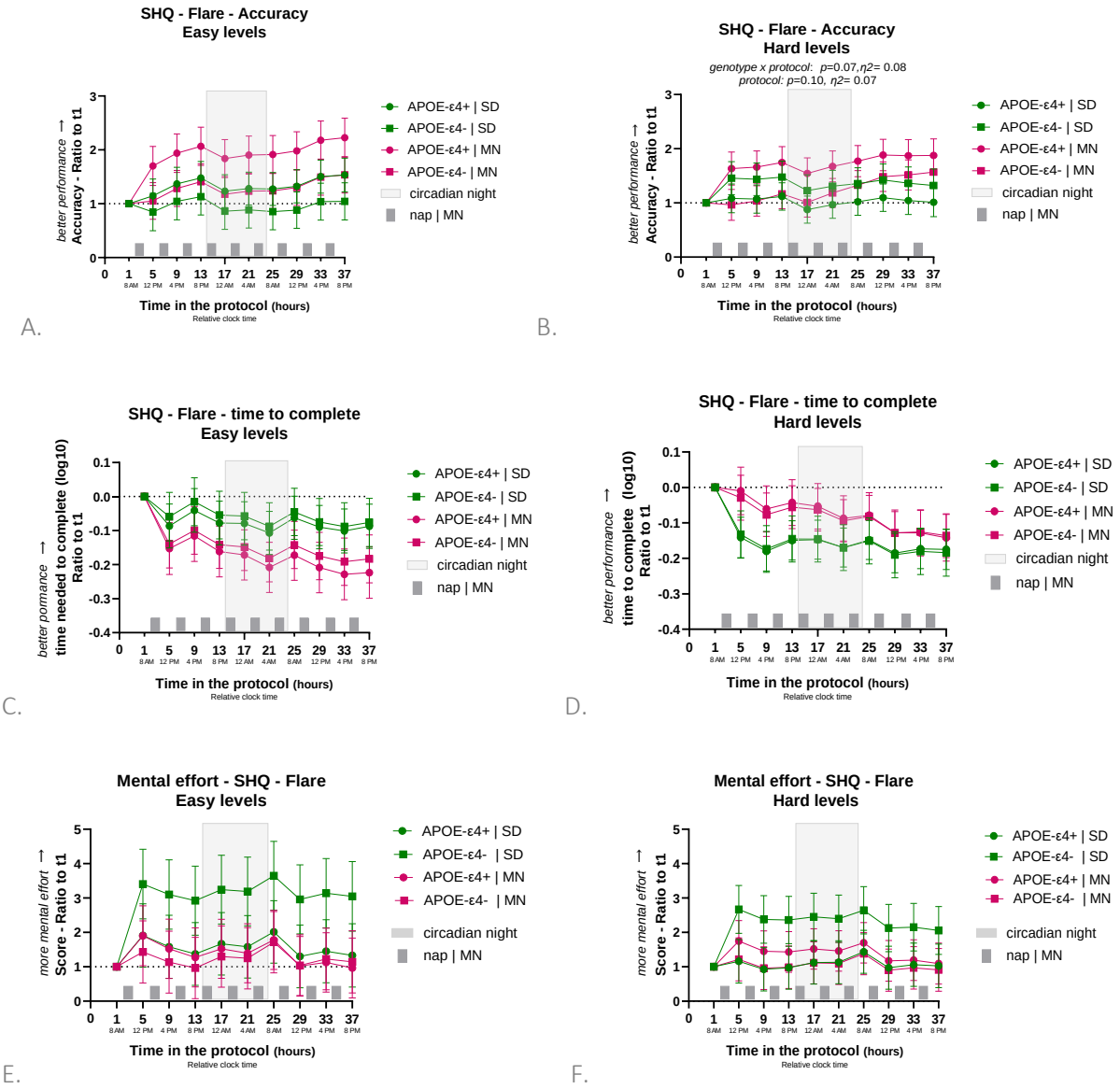


Figure 47. Egocentric navigation performance was assessed by the Sea Hero Quest task (flare levels) throughout 40h of SD and MN protocols. A. Flare – accuracy – Easy levels, B. Flare – accuracy – Hard levels, C. Flare – time to complete – Easy levels, D. Flare – time to complete – Hard levels, E. Flare – subjective Mental effort – Easy levels, F. Flare – subjective Mental effort – Hard levels. Each tick on the x-axis indicated a cognitive assessment and corresponding time in the protocol. The data points are expressed as the least-square means value ±SE.

6.6.5.E. Does objectively measured baseline sleep quality and architecture differ between the low (ε4 allele non-carriers) and high (ε4 allele carriers) genetic risk of AD?

There were no significant differences between ε4 allele carriers and non-carriers in any of the objective sleep quality and macroarchitecture measures at the Baseline Night besides, a marginal significance for N2 as a ratio of total sleep time (TST) (p=0.09, d=0.58), where controls (ε3/ε3) spent a higher percentage of TST in N2 compared to ε4 allele carriers (ε4+: 45.49±9.83; ε4-: 50.47±7.16) (Table 29, Figure 48A). Most of the obtained effect sizes were small and only N2 as % of TST (d=0.58), sleep stability (d=0.50) and N2 duration (d=0.48) reached a moderate magnitude of the effect.

	Outcome measures [unit]	APOE-ε4+ (N=18) (M (SD)	APOE-ε4- (N=18) (M (SD)	Total (N=36) (M (SD)	p	p-adj	Effect size
General sleep parameters	Time in bed [min]	507.31 (25.48)	503.58 (26.79)	505.44 (25.84)	0.67	0.98	0.14
	Total sleep time [min]	405.69 (57.81)	413.17 (53.58)	409.43 (55.06)	0.69	0.98	0.13
	Sleep period time [min]	489.75 (21.73)	486.08 (25.08)	487.92 (23.20)	0.64	0.97	0.16
	Sleep efficiency [%]	80.09 (11.81)	81.98 (9.12)	81.04 (10.44)	0.59	0.97	0.18
	Wake after sleep onset [min]	82.28 (51.04)	69.06 (40.10)	75.67 (45.73)	0.39	1.00	0.29
	Sleep onset latency [min]	11.33 (6.72)	11.25 (11.13)	11.29 (9.06)	0.98	1.00	0.01
	Latency to persistent sleep [min]	1.92 (2.48)	2.08 (1.71)	2.00 (2.10)	0.82	0.93	0.08
	Latency to N1 [min]	17.31 (19.72)	19.67 (23.39)	18.49 (21.35)	0.75	0.95	0.11
	Latency to N2 [min]	20.36 (18.68)	15.81 (12.27)	18.08 (15.75)	0.39	1.00	0.29
	Latency to N3 [min]	20.94 (22.12)	15.72 (12.46)	18.33 (17.89)	0.39	1.00	0.29
	Latency to REM [min]	119.75 (68.50)	120.81 (63.32)	120.28 (65.02)	0.96	1.01	0.02
Sleep structure	Wake duration [min]	99.81 (58.78)	85.47 (47.03)	92.64 (52.97)	0.43	1.00	0.27
	N1 duration [min]	60.36 (32.38)	58.81 (31.59)	59.58 (31.54)	0.89	0.98	0.05
	N2 duration [min]	186.44 (51.57)	208.64 (41.05)	197.54 (47.30)	0.16	1.00	0.48
	N3 duration [min]	83.58 (35.95)	77.08 (42.69)	80.33 (39.04)	0.62	0.98	0.17
	REM duration [min]	75.31 (31.62)	68.64 (21.72)	71.97 (26.95)	0.47	0.96	0.25
	N1, % of TST [%]	15.34 (9.43)	14.31 (7.10)	14.83 (8.24)	0.72	0.95	0.12
	N2, % of TST [%]	45.49 (9.83)	50.47 (7.16)	47.98 (8.84)	0.09	1.00	0.58
	N3, % of TST [%]	20.98 (9.31)	18.74 (9.93)	19.86 (9.55)	0.49	0.92	0.23
	REM, % of TST [%]	18.19 (6.52)	16.47 (4.26)	17.33 (5.50)	0.36	1.00	0.31
	Artefacts [min]	1.78 (3.05)	3.86 (9.52)	2.82 (7.05)	0.38	1.00	0.30
Sleep continuity	Number of awakenings [count]	29.39 (11.30)	26.72 (8.42)	28.06 (9.91)	0.43	1.00	0.27
	Awakenings from N1 [ratio]	0.19 (0.12)	0.18 (0.09)	0.19 (0.10)	0.75	0.93	0.11
	Awakenings from N2 [ratio]	0.06 (0.02)	0.05 (0.02)	0.05 (0.02)	0.49	0.95	0.23
	Awakenings from N3 [ratio]	0.03 (0.02)	0.03 (0.03)	0.03 (0.02)	0.50	0.89	0.23
	Awakenings from REM [ratio]	0.08 (0.08)	0.07 (0.05)	0.08 (0.06)	0.46	1.00	0.28
	Sleep stability [ratio]	0.44 (0.09)	0.40 (0.08)	0.42 (0.08)	0.14	1.00	0.50
	Fast sleep stage changes [ratio]	0.05 (0.02)	0.04 (0.01)	0.05 (0.02)	0.43	1.00	0.27
	Deep sleep stage changes [ratio]	0.17 (0.04)	0.16 (0.03)	0.17 (0.04)	0.24	1.00	0.40
	Shallow sleep stage changes [ratio]	0.16 (0.04)	0.14 (0.03)	0.15 (0.03)	0.20	1.00	0.44
	Big deep sleep stage changes [ratio]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.98	0.98	0.01
	Big shallow sleep stage changes [ratio]	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.70	0.95	0.13

Sleep fragmentation	Sleep fragmentation [ratio]	0.07 (0.03)	0.07 (0.03)	0.07 (0.03)	0.40	1.00	0.28
	N1 entries [count]	42.11 (17.88)	40.78 (15.43)	41.44 (16.47)	0.81	0.95	0.08
	N1 fragmentation [ratio]	0.79 (0.28)	0.78 (0.25)	0.79 (0.26)	0.91	0.98	0.04
	N2 entries [count]	61.83 (21.00)	58.06 (15.47)	59.94 (18.28)	0.54	0.93	0.21
	N2 fragmentation [ratio]	0.34 (0.09)	0.29 (0.09)	0.31 (0.09)	0.10	1.00	0.56
	N3 entries [count]	35.89 (18.07)	28.61 (17.36)	32.25 (17.85)	0.23	1.00	0.41
	N3 fragmentation [ratio]	0.48 (0.31)	0.45 (0.31)	0.47 (0.30)	0.76	0.91	0.11
	REM entries [count]	9.61 (5.41)	8.22 (4.25)	8.92 (4.85)	0.40	1.00	0.29
	REM fragmentation [ratio]	0.14 (0.07)	0.12 (0.05)	0.13 (0.06)	0.35	1.00	0.32

Table 29. Sleep architecture at the Baseline Night – a comparison between APOE-ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: independent sample t-test & Cohen's d; Benjamini-Hochberg procedure was applied to calculate adjusted *p* values (*p*-adj). For a definition of the outcome measures please refer to Table 12 in the Methods section.

Further analyses focused separately on the *first* (Table 30) and the *second* part (Table 31) of the Baseline Night's sleep macroarchitecture. This is because the sleep profile shows considerable change throughout a full night's sleep driven by both homeostatic and circadian processes (e.g. decrease of sleep N3, an increase of wake, REM, and sleep fragmentation from the first to the second part of the night) (Dijk & Archer, 2009). In the *first* part of the night, APOE-ε4 allele carriers showed significantly more fragmented N2 sleep in comparison to non-carriers (p=0.04, d=0.70) (ε4+(*M*±*SD*): 0.43±0.12; ε4-(*M*±*SD*): 0.34±0.13) (Table 30, Figure 48B). There was also a trend towards significance for N2 as % of TST (p=0.11, d=0.55) with ε4 allele carriers spending a lower percentage of TST in N2 (ε4+(*M*±*SD*): 41.91±13.71; ε4-(*M*±*SD*): 48.50±10.00). The effect sizes ranged between none to small except for N2 fragmentation (d=0.70), N2% of TST (d=0.55) and N2 duration (d=0.45) where the effect size of genotype differences was of medium magnitude.

	Outcome measures [unit]	*APOE-ε4+ (N=18) (M (SD)*	*APOE-ε4- (N=18) (M (SD)*	*Total (N=36) (M (SD)*	*p*	*p*-adj	*Effect size*
General sleep parameters	Time in bed [min]	253.58 (12.73)	251.69 (13.39)	252.64 (12.91)	0.67[a]	1.00	0.15
	Total sleep time [min]	204.47 (35.07)	205.39 (30.78)	204.93 (32.52)	0.93[a]	1.00	0.03
	Sleep period time [min]	240.97 (13.07)	240.08 (16.53)	240.53 (14.70)	0.86[a]	1.00	0.06
	Sleep efficiency [%]	80.84 (14.32)	81.46 (10.57)	81.15 (12.41)	0.88[a]	1.00	0.05
	Wake after sleep onset [min]	35.81 (33.62)	31.58 (21.48)	33.69 (27.89)	0.66[a]	1.00	0.15
	Sleep onset latency [min]	11.33 (6.72)	11.25 (11.13)	11.29 (9.06)	0.98[a]	1.00	0.01
	Latency to persistent sleep [min]	1.92 (2.48)	2.08 (1.71)	2.00 (2.10)	0.82[a]	1.00	0.08
	Latency to N1 [min]	17.31 (19.72)	19.67 (23.39)	18.49 (21.35)	0.75[a]	1.00	0.11

	Latency to N2 [min]	20.36 (18.68)	15.81 (12.27)	18.08 (15.75)	0.39[a]	1.00	0.29
	Latency to N3 [min]	20.94 (22.12)	15.72 (12.46)	18.33 (17.89)	0.39[a]	1.00	0.29
	Latency to REM [min]	108.65 (51.27)	112.94 (55.48)	110.79 (52.64)	0.82[a]	1.00	0.08
Sleep structure	Wake duration [min]	48.39 (37.00)	42.11 (25.77)	45.25 (31.58)	0.56[a]	1.00	0.20
	N1 duration [min]	24.17 (19.16)	24.31 (14.62)	24.24 (16.80)	0.98[a]	0.98	0.01
	N2 duration [min]	86.56 (30.84)	99.50 (26.15)	93.03 (28.93)	0.18[a]	1.00	0.45
	N3 duration [min]	64.50 (31.91)	58.53 (28.38)	61.51 (29.91)	0.56[a]	1.00	0.20
	REM duration [min]	29.25 (19.12)	23.06 (15.34)	26.15 (17.37)	0.29[a]	1.00	0.36
	N1, % of TST [%]	13.39 (15.06)	12.33 (7.64)	12.86 (11.78)	0.79[a]	1.00	0.09
	N2, % of TST [%]	41.91 (13.71)	48.50 (10.00)	45.20 (12.29)	0.11[a]	1.00	0.55
	N3, % of TST [%]	31.07 (13.66)	28.40 (12.79)	29.73 (13.11)	0.55[a]	1.00	0.20
	REM, % of TST [%]	13.63 (8.05)	10.78 (6.51)	12.20 (7.36)	0.25[a]	1.00	0.39
	Artefacts [min]	0.69 (2.00)	3.11 (9.01)	1.90 (6.55)	0.65[b]	1.00	0.07
Sleep continuity	Number of awakenings [count]	12.06 (5.67)	12.22 (4.97)	12.14 (5.25)	0.93[a]	0.98	0.03
	Awakenings from N1 [ratio]	0.14 (0.11)	0.19 (0.14)	0.17 (0.13)	0.28[a]	1.00	0.37
	Awakenings from N2 [ratio]	0.05 (0.03)	0.05 (0.03)	0.05 (0.03)	0.53[a]	1.00	0.21
	Awakenings from N3 [ratio]	0.02 (0.02)	0.03 (0.03)	0.03 (0.02)	0.47[b]	1.00	0.14
	Awakenings from REM [ratio]	0.08 (0.08)	0.08 (0.07)	0.08 (0.07)	0.91[a]	1.00	0.04
	Sleep stability [ratio]	0.45 (0.09)	0.43 (0.14)	0.44 (0.12)	0.55[a]	1.00	0.20
	Fast sleep stage changes [ratio]	0.05 (0.02)	0.04 (0.02)	0.04 (0.02)	0.67[a]	1.00	0.15
	Deep sleep stage changes [ratio]	0.19 (0.05)	0.18 (0.05)	0.18 (0.05)	0.59[a]	1.00	0.18
	Shallow sleep stage changes [ratio]	0.17 (0.05)	0.16 (0.05)	0.17 (0.05)	0.56[a]	1.00	0.20
	Big deep sleep stage changes [ratio]	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)	0.70[a]	1.00	0.13
	Big shallow sleep stage changes [ratio]	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.84[a]	1.00	0.07
Sleep fragmentation	Sleep fragmentation [ratio]	0.06 (0.04)	0.06 (0.03)	0.06 (0.04)	0.87[a]	1.00	0.06
	N1 entries [count]	17.94 (9.04)	18.39 (8.45)	18.17 (8.63)	0.88[a]	1.00	0.05
	N1 fragmentation [ratio]	0.91 (0.33)	0.86 (0.29)	0.88 (0.31)	0.61[a]	1.00	0.17
	N2 entries [count	35.33 (11.67)	33.22 (12.40)	34.28 (11.92)	0.60[a]	1.00	0.18
	N2 fragmentation [ratio]	0.43 (0.12)	0.34 (0.13)	0.39 (0.14)	0.04[a]	1.00	0.70
	N3 entries [count]	23.56 (11.33)	19.78 (11.66)	21.67 (11.49)	0.33[a]	1.00	0.33
	N3 fragmentation [ratio]	0.52 (0.47)	0.43 (0.31)	0.47 (0.40)	0.49[a]	1.00	0.23
	REM entries [count]	3.44 (3.01)	3.11 (2.91)	3.28 (2.92)	0.74[a]	1.00	0.11
	REM fragmentation [ratio]	0.14 (0.08)	0.15 (0.10)	0.14 (0.09)	0.65[a]	1.00	0.16

Table 30. Sleep architecture from the *first* part of the Baseline Night - a comparison between APOE-ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: a –independent sample t-test & Cohen's d, b –Mann-Whitney U Test & Rank-Biserial Correlation; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj). For a definition of the outcome measures please refer to Table 12 in the Methods section.

In the *second* part of the Baseline Night, APOE-ε4 allele carriers demonstrated marginally lower sleep stability compared to controls (ε3/ε3) (p=0.10, d=0.56) (ε4+($M \pm SD$): 0.43±0.13; ε4-($M \pm SD$): 0.37±0.08) (Table 31, Figure 48C). The largest effect sizes were found for sleep stability (d=0.56), shallow sleep changes (d=0.46), N2 fragmentation (d=0.45), N3 fragmentation (d=0.43), N3 entries (d=0.42) and sleep fragmentation (d=0.42), albeit nonsignificant.

	Outcome measures [unit]	*APOE-ε4+ (N=18) (M (SD)*	*APOE-ε4- (N=18) (M (SD)*	*Total (N=36) (M (SD)*	*p*	*p*-adj	*Effect size*
General sleep parameters	Time in bed [min]	253.72 (12.75)	251.89 (13.41)	252.81 (12.93)	0.68[a]	0.87	0.14
	Total sleep time [min]	201.22 (34.16)	207.78 (30.03)	204.50 (31.87)	0.55[a]	0.98	0.20
	Sleep period time [min]	246.61 (10.93)	244.67 (12.54)	245.64 (11.63)	0.62[a]	0.91	0.17
	Sleep efficiency [%]	79.34 (13.42)	82.51 (11.04)	80.92 (12.21)	0.45[a]	1.00	0.26
	Wake after sleep onset [min]	44.31 (31.53)	36.14 (24.17)	40.22 (28.00)	0.39[a]	1.00	0.29
	Sleep onset latency [min]	0.89 (2.56)	0.97 (4.12)	0.93 (3.38)	0.94[a]	0.96	0.02
	Latency to persistent sleep [min]	1.64 (1.43)	1.44 (1.10)	1.54 (1.26)	0.65[a]	0.86	0.15
	Latency to N1 [min]	13.25 (21.35)	12.17 (12.12)	12.71 (17.12)	0.85[a]	0.94	0.06
	Latency to N2 [min]	2.61 (3.88)	3.06 (7.59)	2.83 (5.94)	0.83[a]	0.95	0.07
	Latency to N3 [min]	31.31 (44.25)	21.31 (43.60)	26.60 (43.57)	0.51[a]	1.00	0.23
	Latency to REM [min]	56.69 (38.21)	45.53 (24.47)	51.11 (32.12)	0.30[a]	1.00	0.35
Sleep structure	Wake duration [min]	51.42 (32.43)	43.36 (29.11)	47.39 (30.65)	0.44[a]	1.00	0.26
	N1 duration [min]	36.19 (17.61)	34.50 (22.70)	35.35 (20.04)	0.80[a]	0.96	0.08
	N2 duration [min]	99.89 (30.63)	109.14 (22.71)	104.51 (26.99)	0.31[a]	1.00	0.34
	N3 duration [min]	19.08 (13.80)	18.56 (19.27)	18.82 (16.52)	0.93[a]	1.00	0.03
	REM duration [min]	46.06 (23.49)	45.58 (18.37)	45.82 (20.78)	0.95[a]	0.95	0.02
	N1, % of TST [%]	18.30 (9.02)	16.50 (9.65)	17.40 (9.25)	0.57[a]	0.97	0.19
	N2, % of TST [%]	48.94 (11.60)	52.75 (9.09)	50.84 (10.45)	0.28[a]	1.00	0.37
	N3, % of TST [%]	10.39 (10.07)	9.14 (8.90)	9.77 (9.39)	0.70[a]	0.87	0.13
	REM, % of TST [%]	22.37 (9.97)	21.61 (7.43)	21.99 (8.67)	0.80[a]	0.94	0.09
	Artefacts [min]	1.08 (2.13)	0.75 (1.29)	0.92 (1.74)	0.57[b]	0.93	0.09
Sleep continuity	Number of awakenings [count]	17.17 (7.82)	14.44 (5.90)	15.81 (6.97)	0.25[a]	1.00	0.39
	Awakenings from N1 [ratio]	0.23 (0.17)	0.17 (0.11)	0.20 (0.15)	0.24[a]	1.00	0.40
	Awakenings from N2 [ratio]	0.06 (0.03)	0.05 (0.03)	0.06 (0.03)	0.48[a]	0.98	0.24
	Awakenings from N3 [ratio]	0.05 (0.06)	0.04 (0.05)	0.04 (0.06)	0.64[a]	0.87	0.16
	Awakenings from REM [ratio]	0.09 (0.08)	0.07 (0.06)	0.08 (0.07)	0.54[a]	1.00	0.21
	Sleep stability [ratio]	0.43 (0.13)	0.37 (0.08)	0.40 (0.11)	0.10[a]	1.00	0.56

	Fast sleep stage changes [ratio]	0.05 (0.02)	0.05 (0.02)	0.05 (0.02)	0.40[a]	1.00	0.28
	Deep sleep stage changes [ratio]	0.16 (0.05)	0.14 (0.03)	0.15 (0.04)	0.37[b]	1.00	0.18
	Shallow sleep stage changes [ratio]	0.15 (0.04)	0.13 (0.03)	0.14 (0.04)	0.18[a]	1.00	0.46
	Big deep sleep stage changes [ratio]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.45[a]	0.97	0.25
	Big shallow sleep stage changes [ratio]	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.57[a]	0.90	0.19
Sleep fragmentation	Sleep fragmentation [ratio]	0.09 (0.04)	0.07 (0.03)	0.08 (0.04)	0.22[a]	1.00	0.42
	N1 entries [count]	24.28 (10.86)	22.44 (9.46)	23.36 (10.08)	0.59[a]	0.90	0.18
	N1 fragmentation [ratio]	0.77 (0.32)	0.78 (0.32)	0.78 (0.32)	0.93[a]	0.98	0.03
	N2 entries [count]	26.94 (11.69)	25.39 (6.14)	26.17 (9.24)	0.62[a]	0.88	0.17
	N2 fragmentation [ratio]	0.28 (0.11)	0.24 (0.07)	0.26 (0.09)	0.18[a]	1.00	0.45
	N3 entries [count]	12.39 (9.22)	8.94 (7.04)	10.67 (8.27)	0.22[a]	1.00	0.42
	N3 fragmentation [ratio]	0.99 (0.66)	0.74 (0.49)	0.87 (0.59)	0.22[a]	1.00	0.43
	REM entries [count]	6.22 (3.83)	5.11 (2.70)	5.67 (3.31)	0.32[a]	1.00	0.34
	REM fragmentation [ratio]	0.15 (0.08)	0.12 (0.07)	0.13 (0.07)	0.31[a]	1.00	0.34

Table 31. Sleep architecture from the *second* part of the Baseline Night - a comparison between APOE-ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: a –independent sample t-test & Cohen's d, b –Mann-Whitney U Test & Rank-Biserial Correlation; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj). For a definition of the outcome measures please refer to Table 12 in the Methods section.

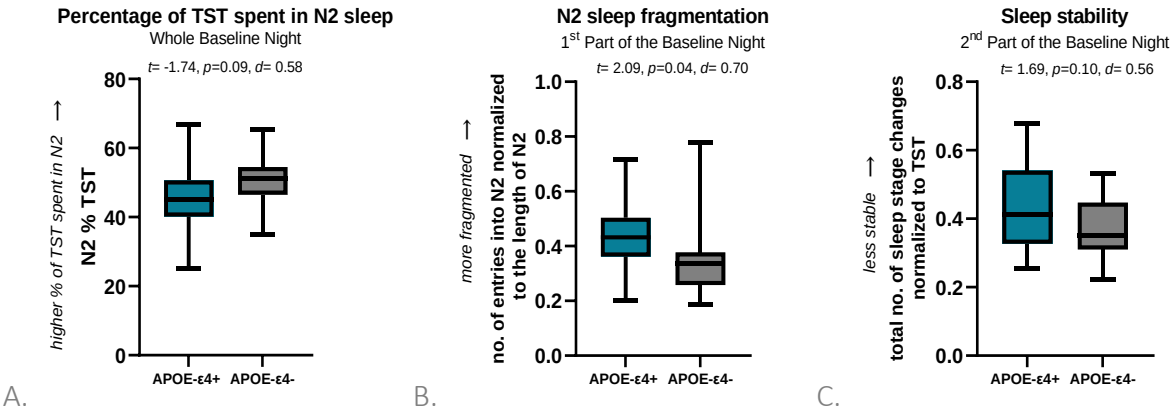


Figure 48. Differences in the Percentage of Total Sleep time spent in N2 (A), N2 sleep fragmentation (B) and Sleep stability (C) for ε4 allele carriers (blue) and non-carriers (grey).

To assess, if ε4 allele carriership modulates the sleep architecture in the *first* and *second* part of the Baseline Night, a mixed model was run (with a repeated statement – *part of the night*) (Table 32). The main effects of the part of the night returned expected results such as a higher percentage of total sleep time spent in N3 sleep in the *first* part of the Baseline Night (p=0.003, η^2=0.24) (1st part($M\pm SD$): 61.51±29.50 min, 2nd part($M\pm SD$): 18.82±16.29 min) and more REM sleep in the *second* part of the

Baseline Night (p=0.04, η^2=0.15) (1^{st} *part*(*M*±*SD*)*:* 26.15±17.13 min*,* 2^{nd} *part*(*M*±*SD*)*:* 45.82±20.49) (Figure 49 A and B). Note, the large effect sizes for the highlighted measures.

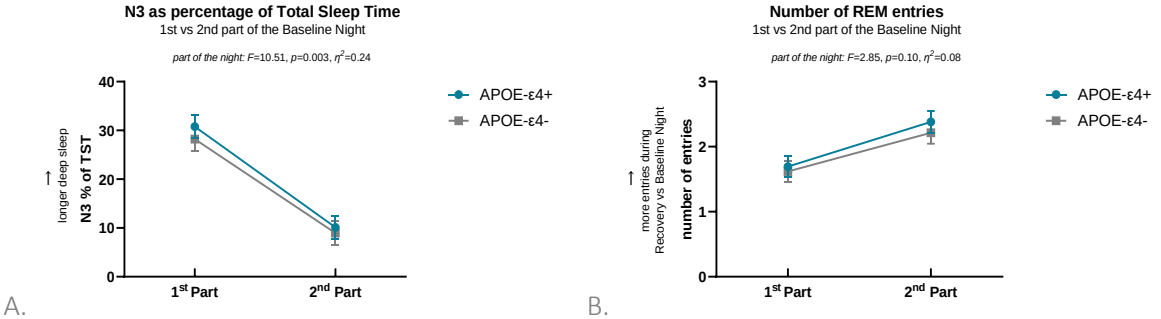


Figure 49. Duration of N3 sleep as a percentage of Total Sleep Time (TST) and a number of REM entries during the first and second part of the Baseline Night in ε4 allele carriers (blue) and non-carriers (grey). Note that raw values are plotted. Data points are expressed as least-square means values ± SE.

While the *part* of the night presented strong significant effects on multiple sleep parameters, there was no significant interaction between ε4 allele status and part of the Baseline Night. Nonetheless, the main effect of the ε4 allele carriership status was marginally significant for N2 fragmentation (p=0.08, η^2=0.06), awakenings from N1 sleep (p=0.10, η^2=0.05) and interaction between awakenings from N1 sleep and part of the Baseline Night (p=0.07, η^2=0.09). Compared to the controls (ε3/ε3), APOE-ε4 allele carriers demonstrated a higher degree of N2 sleep fragmentation in the first part of the night (1^{st}part-ε4-carriers(*LSM*±*SE*): -0.39±0.03*,* 1^{st}part-ε4-non-carriers(*LSM*±*SE*): -0.49±0.03) (Figure 50 A). The N2 sleep fragmentation was on comparable levels in the second part of the night (2^{nd}part-ε4-carriers(*LSM*±*SE*): -0.58±0.03, 2^{nd}part-ε4-non-carriers(*LSM*±*SE*): -0.63±0.03). Further, controls (ε3/ε3) had a higher number of awakenings from N1 over the first part of the night that decreased during the second part of the night (1^{st}part-ε4-non-carriers(*LSM*±*SE*): 0.39±0.04*,* 2^{nd}part-ε4-non-carriers(*LSM*±*SE*): 0.38±0.04) while opposite held for the ε4+ (1^{st}part-ε4-carriers(*LSM*±*SE*): 0.34±0.04*,* 2^{nd}part-ε4-carriers(*LSM*±*SE*): 0.45±0.04) (Figure 50 B).

	Dependent variable (transformation)	Predictors: age			sex			APOE-ε4 status			part of the night			APOE-ε4 status x part of the night		
		F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
General sleep	Total sleep time (TST)	1.20	0.28	0.04	1.03	0.32	0.03	0.03	0.86	0.00	0.25	0.62	0.00	0.25	0.62	0.00
	Sleep period time	0.06	0.81	0.00	0.29	0.60	0.01	0.00	0.98	0.00	2.38	0.13	0.00	0.15	0.70	0.00
	Sleep efficiency (^2)	1.56	0.22	0.05	2.00	0.17	0.06	0.09	0.76	0.00	0.46	0.50	0.01	0.45	0.51	0.01
	Wake after sleep onset (log10)	1.64	0.21	0.05	1.70	0.20	0.05	0.01	0.93	0.00	0.50	0.48	0.02	0.13	0.72	0.00
Sleep structure	Wake duration	3.25	0.08	0.09	1.27	0.27	0.04	0.37	0.54	0.01	2.52	0.12	0.07	1.10	0.30	0.03
	N1 duration (log10)	3.61	0.07	0.10	1.36	0.25	0.04	0.54	0.47	0.01	4.95	0.03	0.13	1.03	0.32	0.03
	N2 duration	0.00	0.98	0.00	2.50	0.12	0.07	1.04	0.31	0.02	1.14	0.29	0.03	0.13	0.72	0.00
	N3 duration	6.40	0.01	0.17	4.89	0.03	0.13	0.49	0.49	0.01	11.25	0.002	0.25	0.32	0.57	0.01
	REM duration	0.30	0.59	0.01	0.85	0.36	0.03	0.63	0.43	0.02	0.59	0.45	0.02	0.39	0.54	0.01
	N1, % of TST (log10)	4.32	0.05	0.12	0.72	0.40	0.02	0.52	0.47	0.01	4.83	0.03	0.12	1.16	0.29	0.03
	N2, % of TST	0.42	0.52	0.01	2.93	0.10	0.08	1.56	0.22	0.03	1.75	0.20	0.05	0.35	0.56	0.01
	N3, % of TST	6.05	0.02	0.16	9.03	0.01	0.22	0.33	0.57	0.01	10.51	0.003	0.24	0.11	0.74	0.00
	REM, % of TST	0.04	0.83	0.00	0.55	0.46	0.01	0.63	0.43	0.01	0.19	0.28	0.02	0.30	0.59	0.00
Sleep continuity	Number of awakenings	0.73	0.40	0.02	1.37	0.25	0.04	0.57	0.45	0.01	4.25	0.05	0.11	1.38	0.25	0.04
	Awakenings from N1 (sqrt)	1.43	0.24	0.04	0.07	0.79	0.00	2.73	0.10	0.05	4.86	0.03	0.13	3.47	0.07	0.09
	Awakenings from N2	0.38	0.54	0.01	0.14	0.71	0.00	0.04	0.84	0.00	0.26	0.61	0.01	0.01	0.93	0.00
	Awakenings from N3 (sqrt)	3.79	0.06	0.10	1.36	0.25	0.04	0.25	0.62	0.01	0.45	0.51	0.01	0.31	0.58	0.01
	Awakenings from REM	1.00	0.32	0.03	3.16	0.09	0.09	0.07	0.79	0.00	0.28	0.60	0.01	0.27	0.61	0.01
	Sleep stability (log10)	1.04	0.32	0.03	3.24	0.08	0.09	0.00	0.95	0.00	0.00	0.96	0.00	0.21	0.65	0.01
	Fast sleep stage changes	0.38	0.54	0.01	0.13	0.72	0.00	0.00	0.99	0.00	0.35	0.56	0.01	0.09	0.76	0.00
	Deep sleep stage changes	4.87	0.03	0.13	3.37	0.08	0.10	0.00	0.96	0.00	0.49	0.49	0.01	0.18	0.67	0.01
	Shallow sleep stage changes	5.02	0.03	0.14	2.44	0.13	0.07	0.00	1.00	0.00	0.38	0.54	0.01	0.15	0.70	0.00
	Big deep sleep stage changes	3.77	0.06	0.11	1.84	0.18	0.05	0.46	0.50	0.01	1.65	0.21	0.05	0.64	0.43	0.02
	Big shallow sleep stage changes	0.45	0.51	0.01	0.46	0.50	0.01	0.00	1.00	0.00	0.09	0.77	0.00	0.09	0.77	0.00
Sleep fragmentation	Sleep fragmentation (log10)	1.17	0.29	0.04	0.54	0.47	0.01	0.34	0.56	0.01	3.00	0.09	0.08	0.93	0.34	0.03
	N1 entries (log10)	1.93	0.17	0.06	1.83	0.19	0.05	0.40	0.53	0.01	3.45	0.07	0.09	0.79	0.38	0.02
	N1 fragmentation	2.10	0.16	0.06	0.01	0.90	0.00	0.30	0.59	0.01	1.21	0.28	0.03	0.30	0.59	0.01
	N2 entries	5.69	0.02	0.15	3.68	0.06	0.10	0.07	0.79	0.06	2.19	0.15	0.00	0.09	0.77	0.00
	N2 fragmentation (log10)	5.42	0.03	0.15	0.00	0.99	0.00	3.23	0.08	0.06	9.55	0.004	0.22	0.93	0.34	0.03
	N3 entries (log10)	7.14	0.01	0.18	0.39	0.54	0.01	0.41	0.52	0.00	5.80	0.02	0.15	0.01	0.91	0.00
	N3 fragmentation	0.71	0.40	0.01	7.32	0.01	0.10	0.14	0.71	0.00	1.14	0.29	0.02	0.00	0.95	0.00
	REM entries (sqrt)	0.39	0.54	0.01	4.84	0.04	0.00	0.00	0.97	0.00	2.85	0.10	0.08	0.10	0.76	0.00
	REM fragmentation (log10)	0.00	0.96	0.00	2.48	0.13	0.07	1.07	0.31	0.02	1.44	0.24	0.04	1.47	0.23	0.04

Table 32. Outcomes of mixed model contrasting First and Second half of the Baseline Night in APOE- ε4 carrier and non-carriers. Statistical test and measure of effect size: Mixed-effects model & eta squared. Used transformation (dependent variable): log10 – log10- transformation, ^2 – power transformation, sqrt – square root transformation. For a definition of the outcome measures please refer to Table 12 in the Methods section.

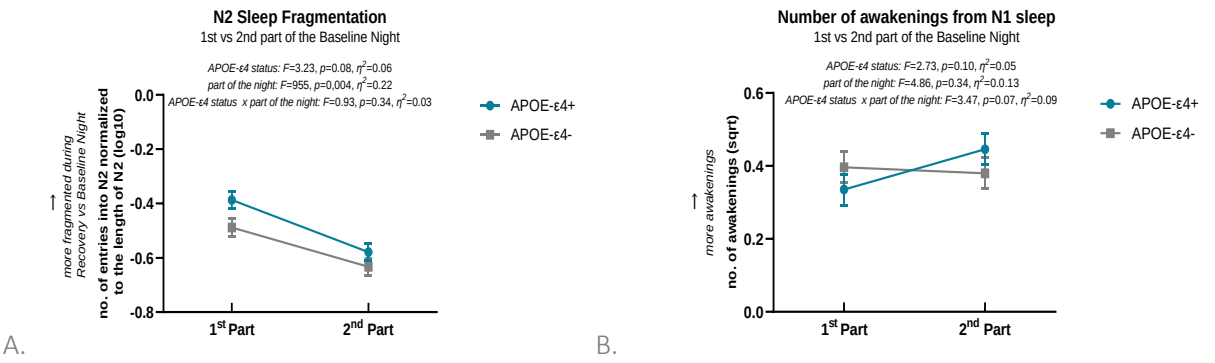


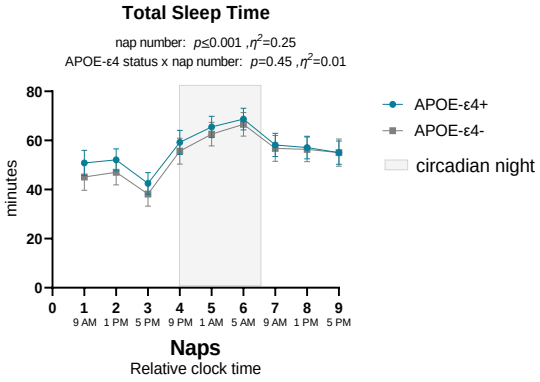
Figure 50. N2 fragmentation and number of awakenings from N1 sleep during the first and second part of the Baseline Night in ε4 allele carriers (blue) and non-carriers (grey). Note that raw values are plotted. Data points are expressed as least-square means values ± SE.

6.6.6. Does the genetic risk of AD (ε4 allele carriership) affect the sleep structure across consecutive naps?

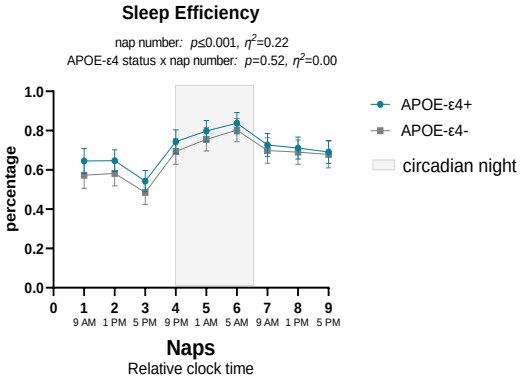
A Fit Linear Mixed-Effects model (lmer) with participants (i.e., (1|id) as a random effect was used to analyse the sleep macro-architecture differences between APOE-ε4 allele carriers and non-carriers across the naps (Model 3)*:* lmer (*variable of interest* ~ age + sex + APOE-ε4 status + nap number + hours of sleep at the Baseline night + nap number*APOE-ε4 status + (1|id)). The tables with detailed statistics for all analysed variables are organized in descending order of the effect sizes and can be found in Appendix 16 - *Statistics - Model 3*.

Importantly, *nap number* had a significant main effect on most of the studied outcome measures and revealed the expected circadian distribution of sleep stages (Appendix 16, Figure 51 A-H). That is, total sleep time was the longest, sleep efficiency was the highest, wake after sleep onset and wake durations were the shortest during the circadian night (Figure 51 A-D). Further, the duration of N3 sleep was the longest during the first part of the circadian night (Figure 51 G), while REM sleep duration was the longest during the early morning hours (Figure 51 H). The latency to N1 and N2 sleep was decreasing throughout the night while being the shortest during early morning hours (Figure 51 I and J). N2 latency was also steadily increasing during the second day of the protocol. Latency to N3 was at a constant level over the protocol, while REM sleep latency was the shortest during the evening of the first day and early morning hours. Further, the effect sizes ranged from none to large with the largest being obtained by the nap number. Notably, APOE-ε4 status and its interaction with the nap number were characterized by the lowest effect sizes. APOE-ε4 status reached the largest effect sizes for REM fragmentation (η^2=0.14), N1 duration (η^2=0.10), wake duration (η^2=0.06), number of awakenings from REM (η^2=0.05) and N3 fragmentation (η^2=0.05), while the APOE-ε4 status*nap number for REM fragmentation (η^2=0.11). Note that the provided nap analyses demonstrate that participants in the

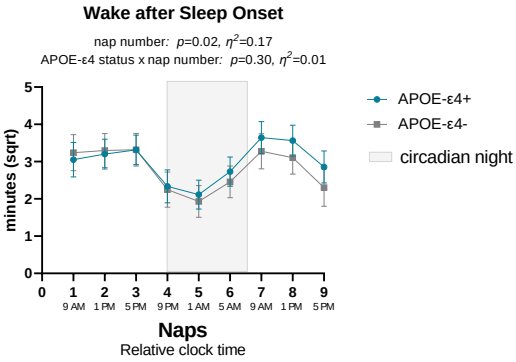
MultiNap experimental condition slept which reveals that the MultiNap protocol was successful at decreasing sleep pressure.



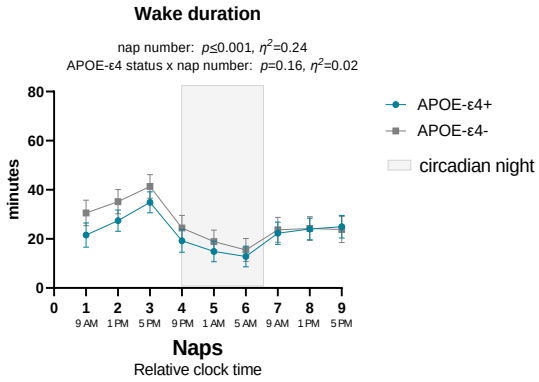
A.



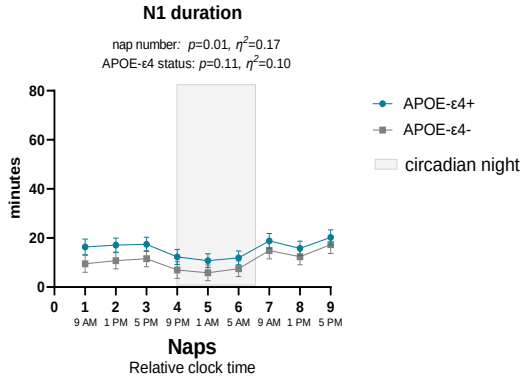
B.



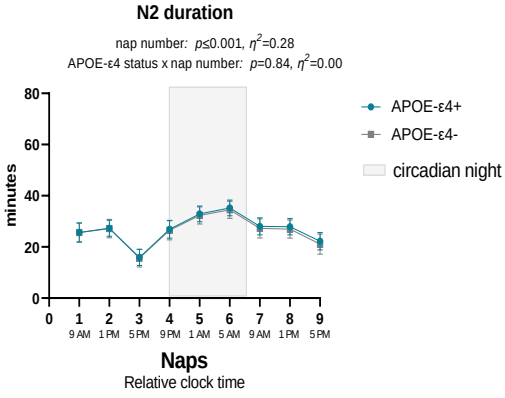
C.



D.



E.



F.

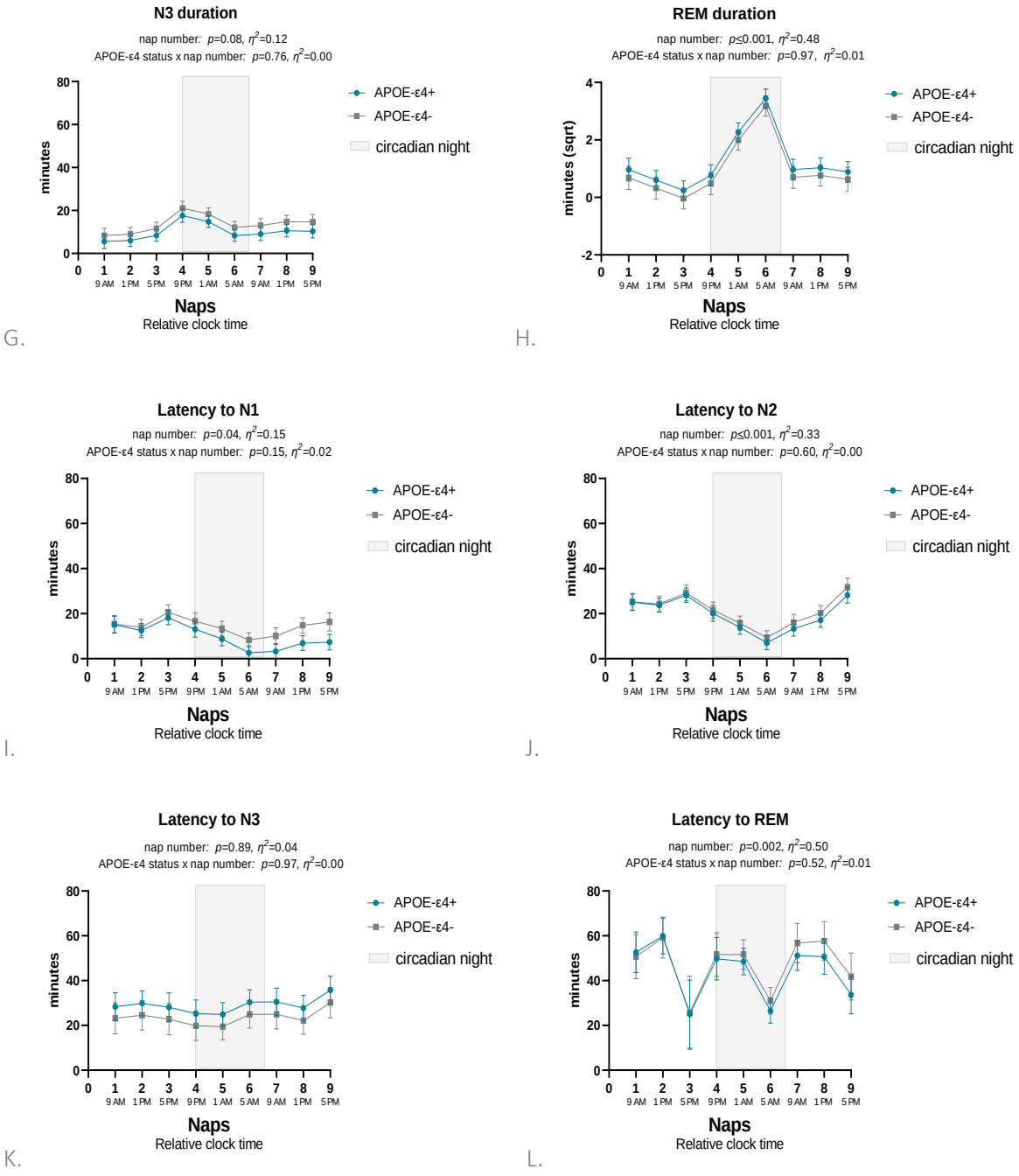


Figure 51. Sleep macro-architecture across 40 hours of MultiNap protocol – comparison between ε4 allele carriers and non-carriers. The X-axis shows nap numbers and corresponding time, while the y-axis indicated time spent in each sleep stage, in sleep overall or wake. (A) duration of Total Sleep Time, (B) Sleep Efficiency, (C) duration of Wake after Sleep Onset, (D) Wake duration, (E) N1 duration, (F) N2 duration, (G) N3 duration, (H) REM duration, (I) Latency to N1 (J) Latency to N2 (K) Latency to N3 (L) Latency to REM. The data points are expressed as the least-square means value ± SE.

Only REM sleep fragmentation measure yielded significant APOE-ε4 status and nap number interaction (p=0.03,η^2=0.13) and main effect of the genotype (ε4+(*LSM±SE*): 0.34±0.10, ε4-(*LSM±SE*): 0.55±0.12) (p=0.05,η^2=0.11) (Figure 52). Compared to controls (ε3/ε3), APOE-ε4 allele carriers had lower REM sleep fragmentation during the first four naps (9 AM to 9 PM – day one) and then higher

fragmentation over the three last naps (9 AM-5 AM - day two). Overall, REM sleep fragmentation was the highest during afternoon naps over the first day of the protocol, while the lowest during the second part of the circadian night (p=0.004, η^2=0.51). A marginally significant main effect of APOE-ε4 status was found for N1 duration, where carriers demonstrated longer N1 sleep duration across the naps (p=0.11, η^2=0.10) (Figure 51E).

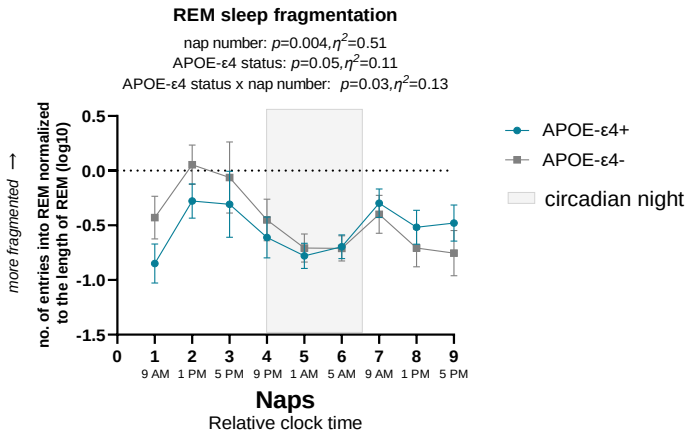


Figure 52. REM sleep fragmentation across 40 hours of MultiNap protocol – comparison between ε4 allele carriers and non-carriers. The x-axis shows nap numbers and corresponding time, while the y-axis indicated time spent in each sleep stage, in sleep overall or wake. Data points are expressed as least-square means values ± SE.

6.6.7. Does the ε4 allele modulate the homeostatic response of sleep duration and architecture to sleep loss (comparison of sleep architecture parameters between Baseline and Recovery Nights)?

To evaluate the effect of sleep pressure modulation, the ratio values were calculated, i.e., Recovery Night outcome measure / Baseline Night outcome measure (e.g., N1 duration at Recovery Night / N1 duration at Baseline Night). The first step was to verify if the sleep pressure modulation protocols worked as expected, hence SD protocol was contrasted with MN (Table 33). As predicted, during the Recovery Night participants who have undertaken total sleep deprivation condition had a significantly longer time in bed (p=0.01, d=1.03) (SD($M\pm SD$): 1.14±0.13, MN($M\pm SD$): 1.04±0.06), total sleep time (p=0.05, d=0.72) (SD($M\pm SD$): 1.19±0.18, MN($M\pm SD$): 1.07±0.15), sleep period time (p=0.002, d=1.21) (SD($M\pm SD$): 1.15±0.12; MN($M\pm SD$): 1.02±0.09) (Figure 53A), and N3 sleep (p=0.001, d=0.64) (SD($M\pm SD$): 3.11±1.91; MN($M\pm SD$): 1.59±0.73) (Figure 53B), shorter REM duration (p=0.03, d=0.78) (SD($M\pm SD$): 1.29±0.36, MN($M\pm SD$): 1.76±0.78) (Figure 53C), and a lower percentage of REM sleep (SD($M\pm SD$):1.07±0.21, MN($M\pm SD$): 1.62±0.62) ($p\leq$0.001, d=0.68), a higher percentage of sleep spent in N3 (SD($M\pm SD$):2.27±1.96, MN($M\pm SD$): 1.47±0.57) (p=0.02, d=0.50), and lower N3 sleep fragmentation ($p\leq$0.001, d=0.81) (SD($M\pm SD$): 0.54±0.25, MN($M\pm SD$): 1.28±0.63) when compared to Baseline Night. Also, at the Recovery Night, latency to N3 sleep was shorter following SD condition (SD($M\pm SD$):0.45±0.62, MN($M\pm SD$):1.86±2.70) (p=0.02, d=0.48) and N3 sleep was less fragmented

compared to MN ($p \leq 0.001$, $d=0.81$) (SD($M \pm SD$):0.54±0.25, MN($M \pm SD$):1.28±0.63). Additionally, during the Recovery Night, individuals in SD protocol had shorter sleep onset latency ($p=0.03$, $d=0.84$) (SD($M \pm SD$): 0.79±0.61, MN($M \pm SD$):1.46±0.94) yet longer latency to persistent sleep ($p=0.01$, $d=0.55$) (SD($M \pm SD$):1.83±2.04, MN($M \pm SD$): 0.42±0.34) and lower number of shallow sleep stage changes ($p=0.03$, $d=0.78$) (SD($M \pm SD$): 0.98±0.17, MN($M \pm SD$):1.17±0.31) relative to the Baseline Night. Note that the listed effect sizes fluctuated between medium and large.

In summary, participants who underwent the SD protocol had more consolidated and longer N3 sleep (i.e. deep sleep) which they entered early on after sleep onset which is a well-established physiological response to increased homeostatic sleep pressure. Longer REM sleep duration following the MN condition suggests lower levels of accumulated homeostatic sleep pressure (i.e., less N3 sleep and more '*space*' for REM sleep).

	Outcome measures	*Sleep deprivation (N=16) (M (SD)*	*MultiNap (N=17) (M (SD)*	*Total (N=33) (M (SD)*	*p*	*p*-adj	*Effect size*
General sleep parameters	Time in bed [min]	1.14 (0.13)	1.04 (0.06)	1.09 (0.11)	0.01[a]	0.03	1.03
	Total sleep time [min]	1.19 (0.18)	1.07 (0.15)	1.13 (0.17)	0.05[a]	0.50	0.72
	Sleep period time [min]	1.15 (0.12)	1.02 (0.09)	1.09 (0.12)	0.002[a]	0.00	1.21
	Sleep efficiency [%]	1.05 (0.17)	1.04 (0.15)	1.04 (0.16)	0.74[a]	1.00	0.12
	Wake after sleep onset [min]	1.44 (1.31)	0.85 (0.62)	1.14 (1.04)	0.22[b]	1.00	0.25
	Sleep onset latency [min]	0.79 (0.61)	1.46 (0.94)	1.14 (0.86)	0.02[a]	0.13	0.84
	Latency to persistent sleep [min]	1.83 (2.04)	0.42 (0.34)	1.15 (1.63)	0.01[b]	0.01	0.55
	Latency to N1 [min]	4.60 (7.92)	1.43 (1.03)	2.97 (5.70)	0.43[b]	0.82	0.17
	Latency to N2 [min]	1.09 (1.22)	1.50 (0.95)	1.30 (1.10)	0.29[a]	1.00	0.38
	Latency to N3 [min]	0.45 (0.62)	1.86 (2.70)	1.18 (2.08)	0.02[b]	0.04	0.48
	Latency to REM [min]	0.80 (0.57)	0.63 (0.31)	0.71 (0.46)	0.31[a]	0.40	0.36
Sleep structure	Wake duration [min]	1.24 (0.98)	0.95 (0.49)	1.09 (0.77)	0.69[b]	0.99	0.09
	N1 duration [min]	0.84 (0.48)	0.94 (0.41)	0.90 (0.44)	0.52[a]	0.53	0.23
	N2 duration [min]	0.85 (0.34)	0.85 (0.17)	0.85 (0.26)	0.95[a]	1.00	0.02
	N3 duration [min]	3.11 (1.91)	1.59 (0.73)	2.33 (1.60)	0.001[b]	0.00	0.64
	REM duration [min]	1.29 (0.36)	1.76 (0.78)	1.53 (0.65)	0.03[a]	0.06	0.78
	N1, % of TST [%]	0.72 (0.41)	0.89 (0.38)	0.81 (0.40)	0.23[a]	0.58	0.43
	N2, % of TST [%]	0.70 (0.19)	0.79 (0.13)	0.75 (0.17)	0.12[a]	0.53	0.55
	N3, % of TST [%]	2.72 (1.96)	1.47 (0.57)	2.08 (1.54)	0.02[b]	0.40	0.50
	REM, % of TST [%]	1.07 (0.21)	1.62 (0.62)	1.36 (0.54)	<.001[b]	0.00	0.68

Sleep continuity	Number of awakenings [count]	1.01 (0.43)	0.86 (0.52)	0.93 (0.48)	0.38[a]	1.00	0.31
	Awakenings from N1 [ratio]	1.54 (1.48)	0.72 (0.61)	1.12 (1.18)	0.04[b]	0.05	0.43
	Awakenings from N2 [ratio]	1.14 (0.52)	1.02 (1.10)	1.08 (0.86)	0.70[a]	1.00	0.13
	Awakenings from N3 [ratio]	0.72 (0.59)	1.03 (1.13)	0.88 (0.91)	0.39[a]	0.68	0.34
	Awakenings from REM [ratio]	1.43 (1.50)	1.00 (0.80)	1.19 (1.16)	0.33[a]	0.73	0.37
	Sleep stability [ratio]	0.93 (0.18)	1.05 (0.27)	0.99 (0.23)	0.16[a]	0.18	0.50
	Fast sleep stage changes [ratio]	1.24 (0.41)	1.27 (0.65)	1.25 (0.54)	0.86[a]	1.00	0.06
	Deep sleep stage changes [ratio]	1.02 (0.19)	1.16 (0.22)	1.09 (0.21)	0.06[a]	0.22	0.67
	Shallow sleep stage changes [ratio]	0.98 (0.17)	1.17 (0.31)	1.08 (0.26)	0.03[a]	0.03	0.78
	Big deep sleep stage changes [ratio]	2.16 (1.31)	2.31 (2.45)	2.23 (1.87)	0.86[a]	0.86	0.08
	Big shallow sleep stage changes [ratio]	1.62 (0.61)	1.61 (0.97)	1.62 (0.80)	0.96[a]	1.00	0.02
Sleep fragmentation	Sleep fragmentation [ratio]	0.86 (0.37)	0.80 (0.48)	0.83 (0.43)	0.69[a]	0.95	0.14
	N1 entries [count]	0.97 (0.35)	0.89 (0.40)	0.93 (0.37)	0.57[a]	1.00	0.20
	N1 fragmentation [ratio]	1.36 (0.60)	1.03 (0.42)	1.19 (0.53)	0.07[a]	0.08	0.64
	N2 entries [count]	1.12 (0.35)	1.16 (0.33)	1.14 (0.33)	0.74[a]	0.78	0.12
	N2 fragmentation [ratio]	1.39 (0.36)	1.42 (0.47)	1.40 (0.41)	0.87[b]	1.00	0.04
	N3 entries [count]	1.39 (0.47)	2.05 (1.72)	1.73 (1.30)	0.15[a]	1.00	0.52
	N3 fragmentation [ratio]	0.54 (0.25)	1.28 (0.63)	0.92 (0.60)	<.001[b]	0.00	0.81
	REM entries [count	1.79 (1.09)	1.98 (1.11)	1.89 (1.09)	0.63[a]	1.00	0.17
	REM fragmentation [ratio]	1.41 (0.85)	1.16 (0.61)	1.28 (0.73)	0.34[a]	0.33	0.34

Table 33. Sleep architecture expressed as ratio of Recovery and Baseline nights – a comparison between APOE-ε4 allele carriers and non-carriers. Statistical test and measure of effect sizes: a –independent sample t-test & Cohen's d, b –Mann-Whitney U Test & Rank-Biserial Correlation; Benjamini-Hochberg procedure was applied to calculated adjusted *p* values (*p*-adj). For a definition of the outcome measures please refer to Table 12 in the Methods section.

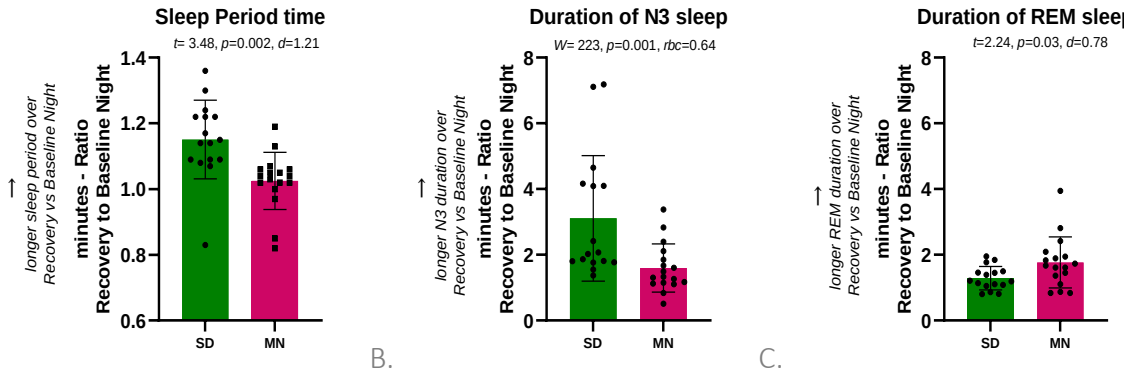


Figure 53. Differences in Sleep Period time (A), N3 sleep (B) and REM sleep (C) during recovery sleep relative to baseline sleep between Sleep Deprivation (SD; green) vs MultiNap (MN; pink) protocols. The values are expressed as a ratio between Recovery Night and Baseline Night.

Once getting reassurance that the experimental sleep pressure manipulation worked, the next step was to investigate if APOE-ε4 allele carriership modulates the homeostatic response of sleep duration and architecture to sleep loss. In ANCOVA controlled for age, sex, APOE status and protocol, the interaction between APOE-ε4 carriership and protocol was explored (Table 34). In line with the literature, age showed a main effect for total time in bed (p=0.08, η^2=0.08), sleep period time (p=0.09, η^2=0.07), N3 duration (p=0.03, η^2=0.11) and N3 as a percentage of total sleep time (p=0.02, η^2=0.13). Protocol differences demonstrated expected changes in sleep physiology that are in line with the results shown in Table 33. APOE-ε4 status had a main effect on a few outcome measures, however, as participants were randomized into either SD or MN conditions and hence underwent different sleep pressure manipulation protocols, the results will be explored only in significant interaction terms.

Several outcome measures revealed a marginally significant main effect of APOE-ε4 status and protocol interaction. APOE-ε4 non-carriers showed the highest percentage of N3 sleep as a percentage of total sleep time following SD protocol (p=0.09, η^2=0.06) (Figure 54A). The post hoc test revealed a significant difference between SD and MN protocols for non-carriers (p_{tukey}=0.02), SD and the marginal difference between non-carriers (SD) and APOE-ε4 carriers (SD) (p_{tukey}=0.09). Further, APOE-ε4 allele carriers had the highest number of awakenings from N1 sleep (p=0.10, η^2=0.08) (Figure 54C). The post hoc test revealed that the difference was statistically significant between carriers in SD and carriers in MN protocols (p_{tukey}=0.05). Total number of awakenings (p=0.06, η^2=0.12) (Figure 54B), fast sleep changes (p=0.08, η^2=0.11) (Figure 54D), number of REM entries (p=0.06, η^2=0.11) (Figure 54E) and REM fragmentation (p=0.07, η^2=0.11) (Figure 54F) demonstrated a trend towards significance yet the post hoc analyses showed no significant differences across analyses pairs. Note that all listed effect sizes had medium magnitude with the total number of awakenings being the largest (η^2=0.12).

	Dependent variable	Predictors: age			sex			APOE-$\varepsilon4$ status			protocol			APOE-$\varepsilon4$ status x protocol		
		F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
General sleep	Time in bed (TIB)	3.38	0.08	0.08	0.07	0.78	0.00	0.08	0.78	0.00	8.99	0.01	0.22	1.37	0.25	0.03
	Total sleep time (TST)	1.86	0.18	0.05	2.56	0.20	0.07	2.07	0.16	0.05	5.19	0.03	0.13	1.55	0.22	0.04
	Sleep period time	3.11	0.09	0.07	0.22	0.64	0.00	0.02	0.89	0.00	12.03	0.002	0.28	1.06	0.31	0.02
	Sleep efficiency	0.02	0.89	0.00	1.61	0.22	0.05	1.01	0.32	0.03	0.15	0.71	0.01	0.06	0.81	0.00
	Wake after sleep onset	0.40	0.54	0.01	0.06	0.81	0.00	4.78	0.04	0.14	2.27	0.14	0.07	0.51	0.48	0.02
Sleep structure	Wake duration	0.44	0.51	0.01	0.19	0.66	0.01	4.22	0.05	0.13	0.86	0.36	0.03	0.21	0.65	0.01
	N1 duration	0.36	0.55	0.01	0.19	0.67	0.01	0.08	0.78	0.01	4.02	0.53	0.01	0.47	0.50	0.02
	N2 duration	0.14	0.74	0.00	0.16	0.69	0.01	4.69	0.04	0.14	0.08	0.78	0.00	0.70	0.41	0.02
	N3 duration	4.96	0.03	0.11	1.54	0.23	0.03	1.68	0.21	0.04	9.69	0.004	0.21	1.86	0.18	0.04
	REM duration	0.29	0.59	0.01	2.27	0.14	0.07	0.04	0.84	0.00	4.85	0.04	0.14	0.06	0.81	0.00
	N1, % of TST	0.01	0.94	0.00	0.01	0.94	0.00	0.29	0.59	0.01	1.38	0.25	0.05	0.23	0.64	0.01
	N2, % of TST	0.13	0.72	0.00	0.44	0.51	0.01	4.73	0.04	0.14	1.95	0.17	0.06	0.00	0.96	0.00
	N3, % of TST	6.48	0.02	0.13	2.08	0.16	0.04	2.98	0.10	0.06	7.05	0.01	0.15	3.12	0.09	0.06
	REM, % of TST	0.07	0.79	0.00	1.77	0.19	0.04	0.05	0.82	0.00	10.99	0.003	0.28	0.00	0.97	0.00
Sleep continuity	Number of awakenings	0.57	0.46	0.02	0.55	0.46	0.02	0.09	0.80	0.00	0.83	0.37	0.03	3.79	0.06	0.12
	Awakenings from N1	1.88	0.18	0.05	0.19	0.70	0.01	0.13	0.72	0.00	4.86	0.04	0.13	2.95	0.10	0.08
	Awakenings from N2	1.05	0.32	0.03	1.13	0.30	0.04	0.97	0.33	0.03	0.10	0.76	0.00	1.25	0.27	0.04
	Awakenings from N3	1.19	0.29	0.04	1.78	0.20	0.07	2.23	0.15	0.08	1.03	0.32	0.04	0.00	0.99	0.00
	Awakenings from REM	0.47	0.50	0.02	0.30	0.59	0.01	1.28	0.27	0.05	0.33	0.57	0.01	0.0 0	0.94	0.00
	Sleep stability	0.01	0.93	0.00	1.37	0.25	0.04	1.15	0.29	0.04	2.36	0.14	0.07	0.36	0.56	0.01
	Fast sleep stage changes	0.51	0.48	0.02	0.13	0.73	0.00	0.15	0.70	0.01	0.03	0.88	0.00	3.28	0.08	0.11
	Deep sleep stage changes	0.86	0.36	0.03	2.16	0.15	0.06	0.08	0.76	0.00	3.91	0.06	0.12	0.00	0.98	0.00
	Shallow sleep stage changes	0.65	0.43	0.02	1.07	0.31	0.03	0.75	0.39	0.02	5.23	0.03	0.15	0.00	0.99	0.00
	Big deep sleep stage changes	0.01	0.93	0.00	0.20	0.66	0.01	0.29	0.60	0.02	0.00	0.96	0.00	1.25	0.28	0.07
	Big shallow sleep stage changes	0.38	0.54	0.01	0.10	0.76	0.00	0.50	0.49	0.02	0.02	0.90	0.00	0.80	0.38	0.03
Sleep fragmentation	Sleep fragmentation	0.07	0.79	0.00	0.21	0.65	0.01	0.50	0.49	0.02	0.14	0.71	0.00	2.72	0.11	0.09
	N1 entries	0.75	0.39	0.03	0.04	0.84	0.00	0.00	0.99	0.00	0.35	0.56	0.01	1.50	0.23	0.05
	N1 fragmentation	0.00	0.95	0.00	0.00	0.98	0.00	0.02	0.89	0.00	3.09	0.09	0.10	0.26	0.62	0.01
	N2 entries	0.00	0.98	0.00	2.62	0.12	0.09	0.34	0.57	0.01	0.10	0.76	0.00	0.40	0.53	0.01
	N2 fragmentation	0.40	0.53	0.01	1.48	0.23	0.04	0.20	0.66	0.01	4.33	0.05	0.13	0.02	0.88	0.00
	N3 entries	0.38	0.54	0.01	0.14	0.71	0.00	0.01	0.91	0.00	2.04	0.17	0.07	1.05	0.32	0.03
	N3 fragmentation	0.00	1.00	0.00	0.21	0.65	0.01	0.53	0.47	0.01	17.36	<.001	0.38	0.72	0.41	0.02
	REM entries	0.72	0.41	0.02	3.89	0.06	0.11	0.01	0.93	0.00	0.26	0.61	0.01	4.02	0.06	0.11
	REM fragmentation	0.11	0.74	0.00	1.41	0.25	0.04	0.00	0.97	0.00	1.04	0.32	0.03	3.66	0.07	0.11

Table 34. ANCOVA outcomes for the sleep architecture outcome measures including the ratio of Recovery and Baseline nights. Statistical test and measure of effect size: ANCOVA & eta^2. For a definition of the outcome measures please refer to Table 12 in the Methods section.

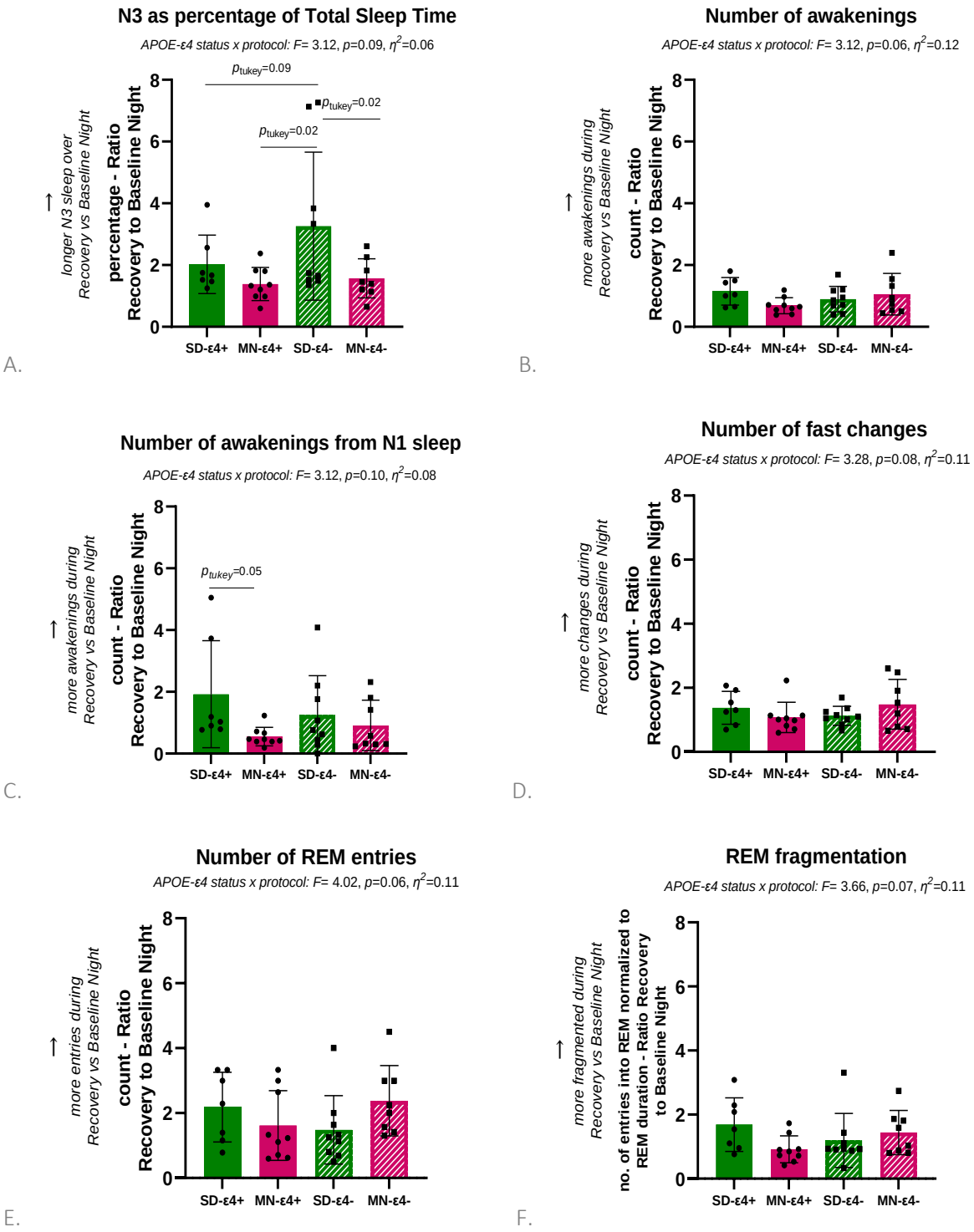


Figure 54. Interactions between APOE-ε4 status and protocol in the context of the homeostatic response of sleep duration and architecture to sleep loss expressed as Ratio of Baseline to Recovery Night. A. N3 as a percentage of Total Sleep time, B. Number of awakenings, C. Number of awakenings from N1 sleep, D. Number of fast changes, E. number of REM entries, F. REM fragmentation

Lastly, by using a Fit Linear Mixed-Effects model (lmer) with participants (i.e., (1|id) as a random effect it was assessed if there is an interaction between APOE-ε4 status, protocol and part of the night (first versus second part), where dependent variables were expressed as a ratio between Recovery and

Baseline night (Appendix 17 – *Statistics – Model 4*). Model 4*:* lmer (*variable of interest* ~ age + sex + APOE-ε4 status + protocol + part of the night + APOE-ε4 status*protocol + APOE-ε4 status*part of the night + protocol*part of the night + APOE-ε4 status*protocol*part of the night + (1|id)). Yet, due small sample size and many predictor variables, the author advises caution while interpreting the results because of possible overfilling (Babyak, 2004) that violates *parsimony* by including more terms than necessary and an overcomplicated approach (using Recovery to Baseline ratio even though justified[69]) (Hawkins, 2004). Therefore, the outcomes can be found in Appendix 17 but are not discussed in the main body of the thesis.

[69] Note, using Recovery to Baseline Ratio limited the model to include 3-way interaction, i.e., APOE- *ε4* status * protocol * part of the night, while including separately Night as Baseline and Recovery would lead to the situation where four-way interaction would need to be involved (i.e., APOE-ε4 status * protocol * part of the night * night) that would significantly complicate interpretation of the results and violate *parsimony* even further.

SUMMARY of FINDINGS – Laboratory session

In both protocols, subjectively (KSS) and objectively (PVT) measured vigilance demonstrated a clear circadian effect with the poorest performance during the circadian night as well as a gradually increasing level of sleepiness (KSS), longer reaction time and higher number of lapses (PVT) in SD compared to MN protocol. Working memory performance (n-back) followed a circadian pattern with worse nocturnal performance and significantly worse accuracy in the SD protocol which was the poorest during the second part of the session (two-back accuracy). Moreover, subjective cognitive demand and mental effort to perform PVT tasks were steadily increasing in SD protocol, while being relatively constant in MN. Importantly, the pattern of cognitive performance in SD and MN protocols strongly suggests that implemented sleep pressure modulation was successful.

The Episodic memory task showed a strong ceiling effect that could have been masked by potential sleep pressure and circadian rhythmicity effects on episodic memory. Further, allocentric navigation performance (SHQ-wayfinding) showed neither circadian nor sleep pressure effects for any of the studied outcome measures besides time to complete easy levels, where the participants who were randomized into the MN protocol were faster, especially during the second day of the session (potentially a learning effect). In the case of egocentric navigation (SHQ-flare), only the accuracy of hard flare levels showed an interaction between the time-of-a-day and protocol, where participants in the MN protocol overperformed those in SD, especially in the second part of the session. The subjective mental effort to complete the SHQ task was relatively constant throughout the cognitive assessments. Interestingly, additional analyses revealed that objective allocentric navigation performance was closely associated with subjective mental effort scores (e.g., longer distance travelled or longer time to complete the level was significantly associated with higher mental effort rating) which was not a case of the egocentric component.

Concerning the modulatory effect of APOE-ε4 status on cognitive assessment, ε4 allele carriers in the SD protocol reported a marginally higher level of sleepiness (KSS) during the first day of the protocol, while those randomized into the MN protocol demonstrated decreasing a number of lapses throughout the second day of the experimental session (PVT). The effect of the APOE-ε4 status carriership was found in neither the n-back nor Episodic Memory tasks. Further, in the case of spatial navigation, no clear APOE modulatory effect was observed besides marginal differences where ε4 allele carriers randomized into the MN protocol had lower time to complete the easy wayfinding levels (during the circadian night), required less time to complete and obtained marginally higher accuracy flare hard levels.

Concerning sleep, during the Baseline Nights, APOE-ε4 allele carriers spent a marginally lower percentage of total sleep time in N2 sleep, had a higher degree of N2 sleep fragmentation during the first part of the night and had slightly poorer sleep stability during the second part of the night compared to non-carriers. Control analyses of Baseline Night sleep macro-architecture revealed an expected longer duration of N3 sleep during the first half of the night and lengthier REM sleep in the second half. Further, no genotype differences were found in the sleep macro-structure across the naps except for the higher level of REM sleep fragmentation in ε4 allele non-carriers. Notably, the nap analyses demonstrated that participants assigned to the MN protocol slept and that the distribution of sleep stages followed circadian rhythmicity with the longest Total Sleep Time during the circadian night, longer N3 sleep during the first part of the night and elongated REM in early morning hours. Furthermore, only REM sleep fragmentation measure yielded significant differences between APOE-ε4 allele carriers and non-carriers. Compared to controls (ε3/ε3), carriers had lower REM sleep fragmentation during the first four naps (9 AM to 9 PM – day one) and then higher fragmentation over the three last naps (9 AM-5 AM - day two).

Analysis of Recovery Nights showed anticipated physiological responses to manipulated sleep pressure such as longer time in bed, lengthier total sleep time, longer N3 sleep, lower N3 fragmentation, earlier sleep onset and shorter N3 sleep latency during the nights following SD compared to MN protocol. The most striking results showed a modulatory effect of APOE-ε4 allele carriership on the homeostatic response to sleep loss where ε4 allele carriers demonstrated unexpected physiological response to total sleep deprivation (SD) by not spending a significantly higher percentage of total sleep time in deep sleep (N3) compared to their carriers counterparties in MN condition. On the other hand, controls (ε3/ε3) randomized into the SD protocol spent significantly more time in restorative N3 sleep compared to controls (ε3/ε3) who underwent the MN protocol. Moreover, during the Recovery Night following the SD protocol, controls (ε3/ε3) spent a higher percentage of total sleep time in N3 compared to at-risk participants. It was not a case of MN protocol, where no significant differences between genotypes were found.

CHAPTER VII – DISCUSSION

The presented PhD project aimed to investigate the interrelationship between APOE-ε4 allele status, subjective and objective measures of sleep and circadian rest-activity patterns and their associations with spatial navigation performance. Another novel research angle addressed in the presented thesis was to investigate the contribution of sleep-wake homeostasis and intrinsic circadian rhythm (experimentally modulated via increasing sleep pressure in SD and minimalizing it in MN protocol) on sleep and cognition in APOE-ε4 allele carriers and non-carriers with a focus on spatial navigation.

APOE-ε4 status and sleep

APOE-ε4 status and self-reported sleep | Screening session

While controlling for age and sex, APOE-ε4 status showed no independent main effect on self-reported sleep (PSQI, ISI), sleepiness (ESS) and chronotype (MEQ). This is in line with previous reports such as in Drogos et al. (2016) where APOE-ε4 was shown to lead to significantly worse objective sleep quality in absence of subjective sleep complaints. Yet, there are other possible reasons that could contribute to these negative findings. In the presented study, before being invited for the screening session, the participants needed to be eligible based on a phone interview during which several questions screening for sleep disorders and general sleep complaints were asked, such as being diagnosed with a sleep disorder, having a shift work, etc. Hence, the cohort of participants enrolled in the screening session was represented greatly by good sleepers, which could have induced a bias in the presented data. Yet, likewise in our results, Tsapanou et al. (2019) reported that none of the investigated self-reported sleep variables was shown to be associated with APOE-ε4 status. On the contrary, a study by Camargos et al. (2019) demonstrated that APOE status was significantly correlated with global sleep quality, yet, curiously, the highest incidence of reported poor sleep quality was among ε2 carriers compared to ε3 and ε4 carriers. Another study investigating subjective sleep measures across APOE polymorphic groups showed that APOE increases the susceptibility to sleep alterations (Spira et al., 2017). APOE-ε4 homozygotes had 38% higher odds of reporting shorter sleep duration compared to non-carriers. In a subset of participants aged ≥50 years, ε4 carriers had 50% higher odds of reporting difficulties in falling and/or staying asleep compared to non-carriers which brings up the question of whether sleep can be affected by APOE polymorphism differently across a lifespan.

Furthermore, ε2/ε2 and ε2/ε3 carriers had 38% reduced odds of napping compared to ε3/ε3 (Spira et al., 2017). Interestingly, a retrospective assessment of habitual napping behaviour over 5 to 10 years preceding the onset of AD showed that the duration of taken naps interacted with the APOE-ε4

genotype (Asada et al., 2000). Long naps, i.e., exceeding 60 min were related to a higher AD risk across ε4 carriers but not in noncarriers. Short naps, i.e., shorter than 30 min were associated with reduced risk of AD incidents in ε4 carriers. Asada et al. (2000) study highlights an interesting research angle as habitual napping across the lifespan might *modulate* or *disturb* sleep physiology and circadian rest-activity patterns. Overall, lower homeostatic sleep need observed in elderly individuals might be related to less consolidated and shorter nocturnal sleep, whereas decreased daytime circadian wake promotion might favour diurnal naps in older age (Schmidt et al., 2012). Hence, on one hand, naps can be viewed as a visible manifestation of sleep fragmentation that might be related to the development of AD, while, on the other hand, as a sleep pressure *supplementary* modulatory mechanism that might serve as preventive behaviour (if it does not affect circadian rest-activity patterns). More studies on this topic are needed as there is no agreement if habitual napping in older age is harmful or beneficial (Zhang et al., 2020). Yet, a recent study by Palpatzis et al. (2022) demonstrated that daytime napping in middle-aged adults is connected to an elevated risk of developing all-cause dementia in older age regardless of APOE status. Notably, one of the limitations of the presented PhD project was not including analyses of napping behaviour which could be especially interesting in the case of conducting a follow-up study.

APOE-ε4 status and habitual rest-activity patterns | Actigraphy session

Analysis of the actigraphy-based rest-activity patterns revealed no significant APOE-ε4 status effects except a decrease in circadian Relative Amplitude (RA) and lower stability of activity-rest patterns in carriers. These associations were not reported yet in the context of cognitively intact APOE-ε4 carriers. The rest-activity stability was suggested to be higher in older age (Luik et al., 2013), whereas significantly lower in individuals with dementia (Van Someren et al., 2019, Saito et al., 2018). Further, the reduced circadian amplitude was reported in patients with dementia (Weissova et al., 2016, Van Someren et al., 2019, Wams et al., 2017), while the magnitude of its decrease was shown to be associated with AD severity (Witting et al.,1990). Moreover, Musiek et al. (2018) reported that increasing age was associated with a decline in rest-activity amplitude and more fragmented 24-h activity rhythms even in absence of preclinical Alzheimer's. Interestingly, independently of age, preclinical amyloid levels were associated with more prominent circadian fragmentation but no further reduction in circadian amplitude. This in turn implies that ageing processes and AD pathology are driven by distinct circadian dysfunctions. The authors proposed that circadian alterations can herald the symptomatic onset of AD. This is in line with research by Tranah et al. (2011) who reported that circadian activity rhythm amplitude reduction was associated with an elevated risk of developing cognitive impairment in cognitively intact elderly women. Notably, it is not clear to what extent circadian rest-activity changes are related to ageing processes, yet a growing body of evidence suggests that 24-h activity rhythms alterations can be involved in age-related diseases (Feijter et al., 2020).

Furthermore, no effects of APOE-ε4 allele status on self-reported sleep quality and sleep efficiency were found when analysing sleep diary outcome measures. That is in line with reviewed studies focused on the impact of APOE genotype where sleep-diary-based outcome measures were reported (Hawang et al., 2018, Lysen et al., 2020).

APOE-ε4 status versus challenged sleep-wake homeostasis | Laboratory session

Participants involved in this PhD project were healthy elderly good sleepers. The subjects who underwent screening sessions were invited based on the telephone interview that aimed to select as suitable participants as possible. Then, the participants enrolled in the actigraphy and lab sessions were chosen even more selectively, i.e., their general health, mental health, sleep and rest-activity patterns needed to meet *all* the eligibility criteria. It was done because the study aimed to investigate the effect of the APOE genotype while trying to minimize the confounding effects of additional risk factors. Therefore, the cohort of the enrolled participants cannot be generalized to the general population. These strict entry requirements were, however, necessary to assess if APOE-ε4 allele carriership will impact sleep macro-architecture and response to sleep loss which in turn required participants whose sleep was *healthy* enough to start the protocols without sleep debt, to be able to fall asleep and maintain sleep and who do not keep falling asleep due to excessive daytime sleepiness caused by, for example, obstructive sleep apnoea. Taking it all into consideration, comparisons with other studies investigating the impact of APOE polymorphism on sleep are challenging because of significant discrepancies between used inclusion and exclusion criteria. For instance in Camargos, et al. (2019) and Lysen et al. (2020) studies participants with comorbidities such as diabetes, hypertension, depression and possibly sleep apnoea were included which is puzzling considering the complex relationship between sleep, cardiovascular health and metabolism. Further, most of the studies on associations between APOE and sleep have not controlled for sleep breathing disorders otherwise than via questionnaires. Sleep breathing disorders are common among the elderly and are characterised by decreased total sleep time and sleep efficiency, more fragmented sleep, and increased daytime sleepiness and naps. Therefore, some of the results could be attributed to sleep disorders such as obstructive sleep apnoea and not to the APOE polymorphism per se. Notably, several studies showed that APOE-ε4 allele carriers are at a higher risk of developing obstructive sleep apnoea, however, the results up to date are inconclusive (Bliwise, 2002, Lu et al., 2016).

Another challenging aspect of placing the results of this PhD thesis into a context of studies investigating the interrelationship between APOE polymorphism and sleep and rest-activity patterns is that the results are highly inconsistent which can be greatly attributed to methodological differences. Some of the subjective sleep assessment methods were limited to only one sleep-related item like in

Burke et al. (2016), whereas the others (Petit et al., 2017, Tsapanou et al., 2019) used a set of sleep-related questions that make a comparison across studies more challenging. Inconsistent results could be further attributed to differences in defining APOE status contrasts across the studies. For example, in Tranah et al. (2018) study APOE status was grouped according to the number of ε4 alleles (0,1,2), whereas, in Drogos et al. (2016) participants were split according to being ε4 allele carriers and non-carrier also including the APOE ε2/ε4 genotype that was excluded in Kahya et al. (2017) and Tsapanou et al. (2019) because of the opposite effect of these two alleles.

Further, although the gold standard for measuring sleep is polysomnography, the number of PSG studies investigating the impact of APOE status on sleep architecture is very limited. In the current study, APOE-ε4 allele carriers were shown to spend a marginally lower percentage of total sleep time in N2 sleep, had a higher degree of N2 sleep fragmentation during the first part of the night and had slightly poorer sleep stability during the second part of the night compared to non-carriers. The results concerning N2 sleep percentage are in line with Drogos et al. (2016) where ε4 allele carriers had a lower percentage of TST in N2. Yet, their study also reported shorter sleep duration, longer WASO, and lower SE in carriers compared to non-carriers which are consistent with impaired objective sleep quality in the risk gene carriers. Overall, to better understand the sleep physiological implications of this marginal decrease of N2 in the carriers' group, one should analyse sleep spindles, K-Complexes and perform spectral analyses, which can provide a better insight into the key micro-structural hallmarks of N2. Reduced N2 and higher fragmentation of N2 could be linked to fewer sleep spindles which are known to be protective for sleep continuity and could have implications for the cognitive function of sleep, e.g. memory (Urakami et al., 2012, Schabus et al., 2006). Also, a reduction of fast spindles was reported in AD and MCI patients compared to the controls (Gorgoni et al., 2016), while lower amplitude and faster frequency of spindles was suggested to be a sleep marker of a greater risk of cognitive decline in older adults (Taillard et al., 2019).

Furthermore, the important message from Drogos et al. (2016) pilot study using home-PSG is that the impact of the ε4 allele carriership on objective sleep quality can herald subjective sleep complaints. It is an interesting notion considering the long asymptomatic yet already Aβ positive phase in AD pathology. It was shown that aggregation of Aβ plaques within the brain is associated with increased sleep disturbances in patients with AD (Yesavage et al., 2004) and that in non-demented participants cerebral amyloid pathology was associated with APOE genotype (Jansen et al., 2015). Interestingly, the age at which 15% of cognitively intact participants were amyloid positive was around 40 years for APOE-ε4ε4 carriers, 50 years for ε2/ε4 carriers, 55 years for ε3/ε4 carriers, 65 years for ε3/ε3 carriers, and 95 years for ε2/ε3 carriers (Jansen et al., 2015).

Most of the presented studies focused on elderly participants, however, the involvement of younger adults is crucial for establishing early sleep and circadian changes related to initial Aβ accumulation and how those changes are associated with the APOE status. Ju et al. (2013) demonstrated that amyloid deposition in cognitively asymptomatic middle-aged individuals was associated with worse sleep quality yet not sleep quantity. Increased sleep fragmentation in the elderly without dementia was associated with a higher risk of developing AD (Lim et al., 2013, Chylinski et al., 2021). Notably, efficient sleep consolidation seems to reduce the detrimental effect of the APOE-ε4+ genotype on incident AD and neurofibrillary tangle pathology (Lim et al., 2013). This moderating role of APOE is further supported by Grau-Rivera et al. (2020) study which showed that insomnia had a more detrimental impact on brain structure among ε4 carriers compared to noncarriers. Yet, a large longitudinal study involving 397,777 participants showed that the effects of sleep duration, insomnia and daytime napping were similar across APOE genotypes (Palpatzis et al., 2022). The study suggests that too short, too long sleep and daytime napping in middle-aged adults is associated with a higher risk of developing all-cause dementia in older age regardless of APOE status. A study conducted by Lucey et al. (2021) supports this U-shaped association demonstrating that in elderly adults cognitive decline was associated with either too much or too little duration of total sleep time, time in N2 and N3, time in REM and NREM slow wave activity. Note that in the presented PhD project, both, ε4 allele carriers and non-carriers reported to sleep on average ~7 hours per night which places them at the bottom of the risk curve. It is possible that moderation in hours spent asleep and balance between sleep stages is crucial for healthy sleep. Palpatzis et al. (2022) demonstrated as well a non-linear, inverse U-shaped relationship between changes in a Preclinical Alzheimer's Cognitive Composite (PACC) score and NREM and REM duration.

Analyses of the naps in the presented laboratory study (MN protocol) did not show any significant differences in sleep macrostructure between carriers and non-carriers besides APOE-ε4 allele carriers showing lower REM sleep fragmentation during the first four naps and then higher fragmentation over the three last naps compared to non-carriers. Notably, in both genotypes, the distribution of sleep stages followed the known circadian rhythmicity and homeostatic sleep regulation with the shortest sleep latency, longest Total Sleep Time and REM sleep during the circadian night, and N3 (deep) sleep dominating the first part of the sleep episodes which suggests well-maintained sleep regulatory control.

One of the remaining questions is whether the associations between APOE polymorphism and sleep and rest-activity patterns change across the lifespan. According to the APOE Antagonistic Pleiotropy Hypothesis, even though ε4 allele carriership can be beneficial in early life, it may increase the risk of cognitive decline only in later life (Tuminello & Han, 2011). A recent longitudinal study supported this hypothesis stating that APOE-ε4 allele carriership in both homozygotes and

heterozygotes is associated with accelerated cognitive ageing yet only in older age (Gharbi-Meliani et al., 2021). Relatively to ε4-non-carriers, poorer cognitive performance was detectable from 65 years of age in APOE-ε4 homozygotes and age 75 years in heterozygotes. Yet, strikingly, APOE-ε4 heterozygotes demonstrated better global cognitive performance compared to ε4 allele non-carrier up to the age of 55 years. Further, ε4 carriers had an elevated risk of developing dementia at an older age. These findings might be associated with *cognitive reserves* as, for instance, the effect of years of education was shown to be protective in elderly ε4 allele non-carriers yet its protecting effect was reduced in ε4 carriers and diminished by a progression of AD (Li et al., 2020). APOE-ε4 allele carriership might increase the neurophysiological vulnerability in older age leading to *accelerated ageing* irrespectively of any neurological compensatory mechanisms. In support of this hypothesis, Sun et al. (2020) reported that compared to non-carriers, ε4 allele carriers demonstrated *steeper age-related decline* over the age of 50 years in such domains as executive functions, attention, language and global cognition (all participants were cognitively intact). APOE-ε4 carriers showed also age-related accelerated reduction of white matter fibres in the left and right hippocampus, right superior longitudinal fasciculus and forceps minor.

Overall, the underlying association between APOE-ε4 status, cognitive decline and their interrelationship with sleep are not well understood. Yet, APOE-ε4 carriers were reported to reach an abnormally elevated level of neocortical Aβ at 63 years of age, while non-carriers fifteen years later, at the age of 78 (Burnham et al., 2020). Also, in elderly adults, PET-assessed Tau protein uptake in the entorhinal cortex and hippocampus was elevated in APOE-ε4 carriers independently of Aβ, sex, age and overall clinical status (Therriault et al., 2020). Importantly, sleep, especially N3 characterized by an increased functioning of the glymphatic system allows the brain the removal of accumulated Aβ and tau tangles (Payne & Walker, 2008, Walker, 2009, Xie et al., 2014). Importantly, even one night of total sleep deprivation was shown to increase the Aβ production in middle-aged adults as sleep deprivation was shown to rise overnight Aβ-38, Aβ-40, and Aβ42 levels by 25–30% compared to sleeping controls (Lucey et al., 2019). One of the main results of the presented thesis was an unexpected physiological response to total sleep deprivation in ε4 allele carriers. This lack of clear N3 rebound might suggest that ε4 allele carriers have affected recovery processes and cannot fully regenerate after a prolonged lack of sleep. Chronic insufficient sleep duration or poor sleep quality can diminish the clearance of Aβ and tau accumulated during wakefulness and, hence, contribute to the development of AD (Holth et al., 2018).

Additionally, only one of the reviewed studies on APOE status and sleep reported N3 sleep changes, where APOE-ε4 homozygotes had increased stage N3 duration compared to ε 3/ε4 carriers (Tranah et al., 2018). These unexpected results might be related to higher *tiredness* as increased slow-wave sleep is a classic marker of increased sleep pressure accumulated during the day, which may be

due to an accelerated build-up of sleep debt during the day or a carry-on effect from previous nights of insufficient sleep duration or its poor quality (Dijk et al., 1990).

APOE-ε4 carriership, spatial navigation and self-reported sleep

APOE-ε4 carriership, spatial navigation and self-reported sleep | Screening session

In the screening cohort, no significant differences in the Virtual Supermarket task (VST) performance were found between APOE-ε4 allele carriers and non-carriers. Analysis of the VST has not demonstrated navigational deficits consistent with what had been reported by Coughlan et al. (2020) and Coughlan et al. (2020) where the authors were able to differentiate between APOE-ε4 carriers and ε4 non-carriers based on their navigation performance in VST and SHQ tasks. That is, at-risk participants performed worse in the egocentric orientation sub-task (VST-E) and favoured navigating along the borders. In the presented PhD project, due to time concerns, only seven out of fourteen VST trials were included in the cognitive test battery. This in turn did not allow measuring the border effect reflecting central vs. boundary preference as trials 1-7 were limited to only half of the supermarket layout and the border response coordinates are reported as the distance from the centre of the supermarket plan. Also, egocentric navigation performance could have been affected by a reduced number of assessed trials which makes a comparison with Coughlan et al. (2020), and Coughlan et al. (2020) studies challenging.

No differences between the two genetic groups were evident in the *general* cognitive assessment which is in line with Coughlan et al. (2020). Furthermore, heading direction (VST-HD) and allocentric navigation (VST-A) were associated with the speed of processing (SDMT), visuospatial attention (TMT-sec) and visuospatial skills and memory (ROCF) which all serve as well-established neuropsychological tools due to their sensitivity to cognitive alterations. This, in turn, supports further the use of VST as a potential screening tool for early cognitive impairment.

Furthermore, no *significant* associations between the two genetic groups were evident between self-reported sleep, sleepiness and chronotype measures and spatial navigation (VST) or any other cognitive outcome measure besides verbal memory (Hopkins - recognition). This can be attributed to the fact that based on PSQI score, the participants were on average characterised as good sleepers with self-reported number of seven hours asleep per night, normal level of daytime sleepiness (ESS), low frequency of clinically relevant insomnia complaints (ISI) and mostly intermediate chronotypes (MEQ). Hence, the variance in the data was relatively low which is not representative of the general population where the scores would be much more diverse which could reveal a potential association between, sleep complaints and spatial navigation alterations. For instance, obstructive sleep apnoea

that leads to sleep fragmentation and is also associated with an increased risk of AD (Bubu et al., 2019) was shown to affect spatial navigational performance in cognitively intact elderly individuals who demonstrated lower overnight maze competition time compare to controls without obstructive sleep apnoea (Mullins et al., 2021).

Notably, the observed trend between a lower number of hours asleep and worse allocentric response (VST-A) for APOE-ε4 non-carriers found in the discussed PhD thesis encourages further investigation. As shown by, for example, Ferrara et al. (2008) or Ferrara et al. (2006) sleep enhances newly encoded spatial memories, while sleep deprivation leads to performance impairment. Spatial memory, which has been extensively investigated in the context of sleep (e.g., Simon et al., 2021, Nguyen et al., 2013, Peigneux et al., 2004, Orban et al., 2006), is critical to navigating in space and its alteration can affect allocentric navigation performance. Yet, exploring the underlying causes of reduced self-reported sleep duration would be critical to understanding this trend better. Reduction of self-reported sleep might be related to undiagnosed obstructive sleep apnoea or paradoxical insomnia, stress, menopausal changes or perhaps ageing processes as, for instance, age-related reduction in deep sleep can give the impression of less refreshing, hence shorter sleep. Although, if the reduction of sleep duration is related to neurodegenerative processes, it could possibly uncover the underlying mechanisms that link it with worse allocentric navigation.

APOE-ε4 carriership, spatial navigation and circadian rest-activity pattern | Actigraphy session

Spatial navigation measured by the Visual Supermarket Task (VST) showed several associations with circadian outcome measures[70]. The relationships between higher circadian amplitude and better VST performance provided an interesting message that the higher the amplitude of 24-h activity rhythms amplitude is, the better heading (VST-HD) and allocentric responses are (VST-A). The relationship between better allocentric navigation and lower Interdaily Stability is counterintuitive as it suggests that higher day-to-day activity-rest pattern consistency reflecting healthier, well-consolidated circadian rhythmicity is associated with worse allocentric spatial navigation. For instance, in a big longitudinal Rotterdam Study, a less stable 24-hours activity pattern predicted all-cause mortality suggesting that disrupted circadian activity can be an early indicator of compromised health (Zuurbier et al., 2014). Consistent with these findings lower circadian stability was also reported in patients with dementia (Van Someren et al., 2019, Saito et al., 2018). More studies on larger sample sizes would be needed to clarify these unexpected results.

[70] Note that due to the small sample size, not all correlations survived corrections for multiple testing, hence to some extent, the discussed results should be treated more like pilot data. Besides, correlational analyses highlight only the statistical relationship between the variables making them very limited in nature.

Additionally, as various age-related diseases are accompanied by circadian rest-activity alteration (Feijter et al., 2020) and spatial navigation deficits might serve as an early marker of preclinical AD (Coughlan et al., 2018), the correlations described in the presented project pave an interesting research path to explore. Based on a growing body of scientific papers linking circadian abnormalities with increased risk for dementia and classifying them as heralds of cognitive impairment (e.g., Musiek et al., 2018, Tranah et al., 2011, Feijter et al., 2020), rest-activity rhythm alterations might serve as an important variable of interest while investigating early cognitive markers of AD such as spatial navigation. Based on extensive work by Musiek, the *quality* of circadian rest-activity patterns can be an important determinant of health and successful ageing.

In the current study, APOE-ε4 allele status demonstrated a modulatory effect on the relationship between actigraphy-measured circadian rest-activity patterns and spatial navigation. Interestingly, all the significant effects were found in the APOE-ε4 non-carriers. Overall, better egocentric navigational performance (VST-E) was associated with lower fragmentation of rest-activity patterns, while better allocentric response (VST-A) was correlated with higher circadian amplitude. As lower rest-activity amplitude and higher fragmentation are commonly associated with ageing[71] (Feijter et al., 2020), one might assume that a better 24 h activity rhythm in older individuals might indicate healthier ageing and be a prerequisite for more efficient allocentric navigation. As lower amplitude and higher fragmentation of the rest-activity rhythmicity were shown to elevate the risk of developing AD in healthy older adults (Li et al., 2020), circadian monitoring tracking chronodisruptions might act as a promising *ageing predictor* (Martinez-Nicolas et al., 2018). In rodents, circadian clock dysfunctions were shown to lead to a neuroinflammatory response, oxidative stress, and neuronal damage (Musiek et al., 2013) which implies that circadian alterations in humans could promote neurodegeneration (Musiek et al., 2016). Given the fact that one of the earliest prodromal cognitive markers of AD is spatial navigation impairment (Coughlan et al., 2018) there can be an association between alterations in circadian rhythmicity and spatial navigation.

Interestingly, subjective sleep measures (sleep diary) showed numerous associations with spatial navigation and APOE-ε4 allele carriership. Higher average sleepiness in the morning (KSS) suggesting unrefreshing sleep was correlated with poorer allocentric (VST-A) and heading (VST-HD) responses. Also higher reported difficulty to get up in the morning was associated with worse allocentric (VST-A) and heading responses (VST-HD). These results suggest that unrefreshing sleep can impact spatial navigation performance which is in line with the research showing navigation impairment in obstructive sleep apnoea patients (Varga et al., 2014, Mullins et al., 2021) whose sleep is characterized

[71] This chronodisruption can be related to age-related light sensitivity (e.g., due to cataract), habitual napping behaviour or a more sedentary lifestyle (that might be associated with mobility issues) (Martinez-Nicolas et al., 2018).

by prominent fragmentation leading to nonrestorative sleep and consequent diurnal sleepiness. However, causal associations cannot be established based on this observational data.

Two initially counterintuitive associations need to be addressed, namely, a correlation between shorter total time in bed (TIB) and better heading direction response (VST-HD) in ε4 allele non-carriers and a significant association between longer nocturnal awakenings were associated with better allocentric response (VST-A) in carriers. Longer TIB might indicate nonrestorative sleep while shorter TIB might suggest more consolidated and refreshing sleep. As indicated by the U-shape association between sleep duration and all-cause mortality or risk of cognitive impairment (Cappuccio et al., 2010, Palpatzis et al., 2022), neither too long nor too short sleep is a good sign. In fact, the majority of the participants who slept a moderate number of hours indicated the best heading response. Regarding the longer self-reported awakenings and better allocentric performance, the data by Lecci et al. (2020) showed that not only individuals with paradoxical insomnia but also healthy controls overestimate subjective sleep latency and underestimate the number of awakenings which suggests that subjective sleep duration might be prone to misperception. Further, patients with paradoxical insomnia overestimated their wake time. Even though we screened for insomnia and daytime sleepiness, a PSG assessment would be needed to screen for paradoxical insomnia, hence we cannot exclude the possibility that some of our participants suffered from it.

APOE-ε4 carriership, spatial navigation and the modulatory effects of sleep pressure and time of the day | Lab session

One of the aims of the project was to examine the effect of sleep pressure and time-of-the-day on spatial navigation and how it differs between APOE-ε4 allele carriers and non-carriers. The novel task assessing spatial navigation – Sea Hero Quest (SHQ) was used for the first time in the context of sleep pressure manipulation and circadian modulation. The experimental protocol required repeated assessments of spatial navigation leading to the situation where a relatively small number of SHQ levels were randomized across ten cognitive assessment sessions. To address the need for repeated assessment during the study preparation phase, the SHQ levels were matched based on their difficulty and divided into *easy* and *difficult* ones based on a pilot study on a cohort of young adults. Further, data from six sleep lab sessions were lost due to technical difficulties. Hence, the presented results involving the task should be treated as a pilot study highlighting methodological challenges and providing recommendations for the forthcoming studies using similar experimental protocols.

Generally, the obtained results suggest that spatial navigation assessed by SHQ does not follow a clear circadian modulation. Further, the observed time of a day effect could be rather attributed to the *learning effect* related to a small number of levels that could have been potentially memorised,

especially in flare levels where the feedback was provided. In fact, the limited number of levels used in this task represented a key factor that affected the exploration of egocentric navigation. That is the small range of response options in the SHQ flare levels, i.e., one, two or three stars reduced greatly the variance in the data. In fact, in Coughlan et al. (2020) study, the SHQ parameter *flare accuracy* was not included in analyses due to its limited categorical variation in response.

Furthermore, based on the literature on the effect of sleep on spatial navigation, one could hypothesise that SHQ performance in MN protocol will be better than in SD. It was shown that brief 1.5h-long naps were beneficial for rout-learning in humans (Wamsley et al., 2010), while a handful of studies demonstrated a detrimental effect of sleep deprivation on spatial memory (e.g., Ferrara et al., 2006, Orban et al., 2006, Ferrara et al., 2008, Rauchs et al., 2008, Nguyen et al., 2013, Simon et al., 2021). Yet none of the reviewed studies contrasted experimentally induced low and high sleep pressure conditions up to date. This in turn highlights an exciting research gap that has been addressed by the current study.

Importantly, subjective (KSS) and objective vigilance (PVT), n-back and mental effort outcome measures showed the expected effect of manipulated sleep pressure that is in line with other studies using a similar protocol to ours (e.g., Graw et al., 2004, Reichert et al., 2017, Cajochen et al., 2001, Maire et al., 2018). In the case of SHQ performance, the potential protocol and/or session number effects could have been undetected due to the relatively small sample size as suggested by small eta^2 effect sizes along insignificant *p*-values in mixed models involving SHQ. On the other hand, due to the cognitive complexity of spatial navigation, it can be potentially viewed as a high-order cognitive function which can make it more resilient to increasing sleep pressure and/or fluctuations induced by circadian rhythmicity.

Further, the rationale behind including the Episodic memory task was to control for memory performance along SHQ as one could argue that potential effects found in spatial performance can be attributed to encoding and retrieval processes. The chosen task, however, showed a prominent ceiling effect and seemed to be too easy for healthy elderly participants. This in turn could have masked sleep pressure and circadian rhythmicity effects on episodic memory as sleep is critical for episodic memory consolidation (Inostroza & Born, 2013), while sleep deprivation was shown to lead to neuronal inability to encode new memories (Yoo et al., 2007).

Lastly, APOE-ε4 status had a very modest modulatory effect on cognitive assessment. APOE-ε4 allele carriers randomized into the MN protocol had a decreasing number of lapses throughout the second day of the experimental session (PVT) which might suggest more refreshing sleep yet this was not confirmed by our sleep analyses. Further, no *clear* APOE modulatory effect was observed in the

context of SHQ performance besides marginal differences where ε4 allele carriers randomized into the MN protocol had lower time to complete the easy wayfinding levels (during the circadian night), required less time to complete and obtained marginally higher accuracy flare hard levels. These results are not in line with previous research where the authors demonstrated a clear negative effect of the ε4 allele carriership on spatial navigation performance as measured by SHQ (Coughlan et al., 2018). Nonetheless, due to the use of different levels of SHQ and very different experimental settings, a direct comparison of the results is very challenging. No effect of the APOE-ε4 status carriership was found in either the n-back or Episodic Memory tasks.

7.2. Limitations

Impact of Covid-19 and lost data

Data collection was affected by three Coronavirus lockdowns in England because of which the data acquisition was stopped for an overall 9 months. Moreover, due to technical issues, SHQ data from six sessions were lost (3 MN and 3 SD protocols), hence spatial navigation results should be viewed as a pilot study that paves the way for future research. The sample sizes of screening and actigraphy sessions were affected to a much smaller extent by the lockdowns.

Limitations related to the age range

Some of the described negative results could be attributed to a too-wide age range compared to the sample size.

Limitations related to the cognitive tasks

Considering extensive cognitive assessment and the use of novel tasks, the following limitations should be addressed:

A) In the Supermarket task, the assessment of only seven out of fourteen levels did not allow for measuring the *border effect.*
B) In the Sea Hero Quest task, a small number of levels[72] could lead to the learning effect, i.e., practice effect contamination, especially in flare levels where the feedback was provided after each trial.
C) The outcome of the flare levels in the Sea Hero Quest task was defined as a number of stars, where three stars was a correct response, while one star was incorrect. This, in turn, has given a very limited range of potential scores and consequently reduced variance in the data.

[72] The levels were matched based on the level of difficulty which was assessed during the pilot study. We have used the map levels having up to 3 checkpoint and flare levels with up to 2 turns.

D) Concerning the Episodic memory task, in the source memory component, the participants could have been confused if a given image was displayed in the current or previous session(s) as all images were automatically randomized for every single assessment and the same stimuli could be displayed in two or more consecutive trials

E) The white background implemented in the Episodic memory task made it hard to navigate a white cursor which made the reaction time (i.e., the time needed to complete) inaccurate. That has not allowed us to investigate if, for example, RT consistency is correlated with measures of episodic memory. A recent study by Hultsch et al. (2022) showed that speed of responding inconsistency was negatively correlated with episodic memory performance and suggested that variability of performance is a significant indicator of cognitive functioning in older adults.

F) Some of the participants happened to be unfamiliar with iPad-based assessments which lead to distress while performing the Sea Hero and Virtual Supermarket tasks.

Limitations related to the field session

A) Sleep dairies have not included sample answers providing a precise format of answers that the participant should have provided, e.g., a participant reported that (s)he woke up five times during the night and then in the questions asking how long were awakening has written '5 minutes' what made it challenging to understand if each of the awakenings lasted 5 minutes or all in total.

B) Not analysing nap data provided in the sleep diary.

C) Not calculating the mean activity level during the 10 most active hours (M10) and the mean inactivity in the 5 least active hours (L5) variables affected the interpretation of found differences in Relative Amplitude (RA = M10 – L5)

Limitations related to the sleep laboratory sessions

A) Lack of adaptation night could lead to the *first night effect* (Agnew et al., 1966)

B) No triggers were implemented in the sleep lab cognitive assessment which made EEG analysis (e.g., Event-Related Potentials and Time-Frequency Analyses) impossible.

7.3. Future directions

Follow-up of presented PhD thesis

The most interesting results originating from the presented PhD thesis that deserve the potential follow-up studies are those demonstrating that APOE-ε4 allele carriers had a marginal reduction in circadian rest-activity amplitude, poorer interdaily stability, decrease in the percentage of TST in N2 at the Baseline Night and the lack of a clear N3 sleep rebound during Recovery Night following

sleep deprivation protocol. If the results are replicated, future research could aim to clarify what are the functional and cognitive consequences of circadian (reduction of relative amplitude and interdaily stability) and N2 sleep alterations, as well as if chronic, insufficient N3 sleep rebound can contribute to the development of AD. Moreover, it would be important to better understand the oscillatory aspects of the highlighted genotype-dependent changes in N2 and the N3 rebound (e.g. sleep spindles and slow wave activity) as they are closely interlinked with cognition. Ideally, these potential future studies would be longitudinal and followed by subsequent intervention studies aiming to improve sleep and circadian changes.

Impact of sleep and circadian rest-activity patterns on spatial navigation – study design

As highlighted in the Introduction – Chapter III, there is a lack of research on the impact of sleep and circadian rhythmicity on spatial navigation as the cited studies were strongly dominated by spatial memory. The presented PhD project has just scratched the surface of this unexplored area of research. Future studies involving repeated measures of spatial navigation should avoid repetition of trials where the feedback is provided to avoid memory effect, e.g., repetition of SHQ flare levels through a 40-hour-long protocol. Also, as timing in circadian protocols is very strict, one could consider the timed spatial navigation assessment to avoid delays related to long competition time.

Acknowledging the complexity of sleep

As revealed in the literature review provided in the Introduction – Chapter II, there is a gap in knowledge regarding sleep micro-architecture across APOE polymorphism. Hence, future investigations should include EEG spectral analyses with the frequency divided into classical frequency bands (delta, theta, alpha, sigma, beta, gamma) or divided into 1, 0.5 or 0.25Hz bins for better resolution. Obtained spectral sleep traits can highlight potential differences in slow oscillations (delta range) or spindle activity (sigma band) that could be further explored by counting the number of sleep spindles and K-Complexes, measuring the amplitude of slow oscillations and K-Complexes as well as analysing slow oscillations-spindle coupling. These micro-sleep analyses can be further motivated by reported alterations in sleep micro-architecture in AD and MCI patients (De Gennaro et al. 2017, Reda et al., 2016, Gorgoni et al., 2016, Mandel, 2020).

It is also important to highlight methodological challenges related to investing sleep in the older population. Even in absence of a neuropathological condition, sleep changes in the elderly compared to young adults are very significant, e.g., advanced bed and wake-up time, more fragile (higher number of awakenings) or shorter and lighter sleep (less deep sleep) (Dijk et al., 2000). However, visual scoring according to R&K or AASM does not provide the sleep staging rules adjusted to the elderly population.

For instance, according to the standardized AASM rules, to score an epoch as N3 sleep, at least 20% of a 30-sec EEG segment must present waves between 0.5-2 Hz with a minimum amplitude of 75 µV. The problem is that slow waves are characterized by significant age-related reduction, hence if one uses a fixed and strict amplitude threshold of 75 µV, a segment will be scored as N2. Hence, even if the epoch consists predominantly of low-mixed frequency EEG background activity, the muscle tone is reduced, eye movement is absent and slow waves are seen but do not meet the amplitude criterium, the segment should not be scored as N3 which blurs the boundaries between N2 and N3 sleep. Hence, while searching for early sleep biomarkers of AD, methodological considerations should be addressed to better define sleep in healthy elderly individuals (Muehlorth & Werkle-Bergner, 2019). One of the promising solutions would be the implementation of personalized sleep staging that acknowledges individual EEG characteristics, especially if used for longitudinal sleep monitoring (Phan et al., 2020).

Composite scores and multi-method investigation of early sleep, circadian and cognitive markers of AD

Investigation of sleep and rest-activity patterns in the context of AD is crucial because most sleep complaints can be successfully treated making sleep disturbances a potentially *modifiable* risk factor for AD (Minakawa et al., 2019). Importantly, sleep and circadian rhythmicity alterations can be attributed to preclinical Aβ accumulation and or/and tau pathology (Wang & Holtzman, 2020, Winer et al., 2020). Therefore, longitudinal studies involving Aβ deposition and tau measurements are essential to better understand their temporal interrelationship with early sleep and rest-activity pattern alterations in AD. Further, Alzheimer's can result in prominent *neuropsychiatric* symptoms (Mendez, 2021), therefore, future studies focusing on early sleep markers of AD should be supplemented with careful neuropsychiatric evaluation because potential sleep disturbances can be comorbid of depression or anxiety which have been also indicated as an independent risk factor for AD (e.g., Burke et al., 2016, Santabárbara et al., 2019).

AD is primarily a *neurocognitive* disorder (Mendez, 2021) yet its early detection based on cognitive assessment is challenging. Subtle, pre-clinical cognitive alterations associated with, gradually increasing levels of Aβ can be overlooked if the neuropsychological tasks are not specific and sensitive enough (Schindler et al., 2017). Therefore, supplementing the studies with the *standardized cognitive composite scores* such as PACC, that involve longitudinal study design would help to track temporal dynamics of investigated cognitive markers, ideally in combination with PET neuroimaging with Aβ tracer or CSF tau/ Aβ42 examination. Another aspect worth consideration in the context of cognitive assessment is the evaluation of *cognitive reserves* (Stern, 2012) as building up cognitive reserves can allow the brain to counter/mask neurological damage more effectively (Larsson et al., 2017). Last but not least, *subjective cognitive decline* in absence of objective cognitive deficits was suggested to be a

potential harbinger of prodromal AD and was shown to be associated with sleep alterations (Tsapanou et al., 2019, Bubbico et al., 2019) making it yet another promising outcome measure.

Overall, the literature suggests that APOE-ε4 polymorphism is only one of many markers increasing the risk of AD. Hence, implementation of *aggravated AD risk scores* involving brain, physical and psychosocial health can provide a more holistic approach to understanding the underlying biological structures and/or physiological functions leading to AD. The Causal Loop Diagram for AD (Uleman et al., 2020, Figure 1) emphasises the need for a multi-methods approach to tackle the complexity and multicausality of AD.

Acknowledging biological sex differences in APOE research

The presented PhD thesis demonstrated several independent sex effects, e.g., a striking difference between N2 and N3 sleep durations across naps (the MN protocol, Appendix 16 - *Statistics - Model 3*) between males and females. Importantly, numerous studies reported that the APOE-ε4 allele interacts with biological sex and modifies the risk of AD. The APOE-ε2/ε3 genotype was shown to have a more protective effect on women, decreasing their risk of AD compared to men (Neu et al., 2018). On the contrary, females who are APOE-ε4 allele carriers were reported to be more likely to develop MCI and AD compared to ε4 positive males (Farrer et al., 1997, Neu et al., 2018) and were shown to be at greater risk of transit from healthy ageing to MCI and from MCI to AD (Altmann et al., 2014). Studies reported a stronger association between APOE-ε4 and CSF-derived Tau levels in females than in men, especially when the participants were Aβ-positive (Altmann et al., 2014, Deming et al., 2018, Hohman et al., 2018). Another study found an association between APOE-ε4 and cognitive decline between ages 70 and 80 years in women exclusively (Lehmann et al., 2006). The culprit of these cognitive changes can be perimenopause to menopause transition and related estrogen loss which is supported by studies highlighting the association between APOE-ε4 carriership, menopause and cognitive decline (Riedel et al., 2016). Notably, APOE-ε4 postmenopausal women between the age of 49 and 69 who stopped hormone replacement therapy had significantly greater telomere loss, i.e., telomer shortening compared to ε4 allele non-carriers which is a sign of accelerated ageing (Jacobs et al., 2013). Therefore, future studies should include information about menstruation, menopause and andropause to facilitate a better understanding of differences related to biological sex and associated hormonal changes.

Sample sizes

Future studies might consider using data biobanks such as UK Biobank to significantly expand investigated sample size which will lead to more accurate results, better use of tax-payers money invested in conducted scientific research and give a new life to already collected data.

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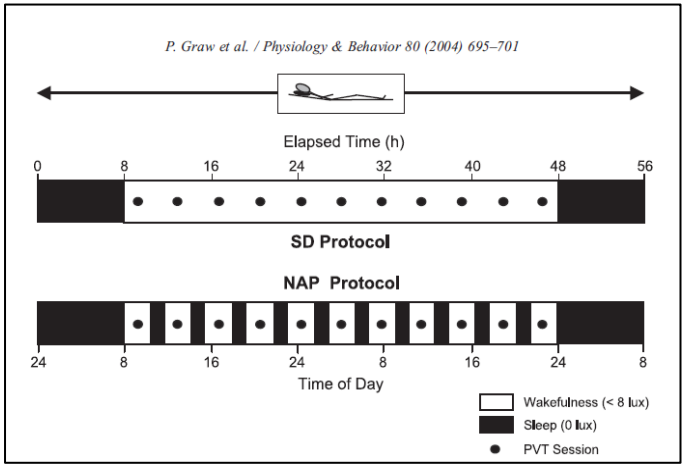
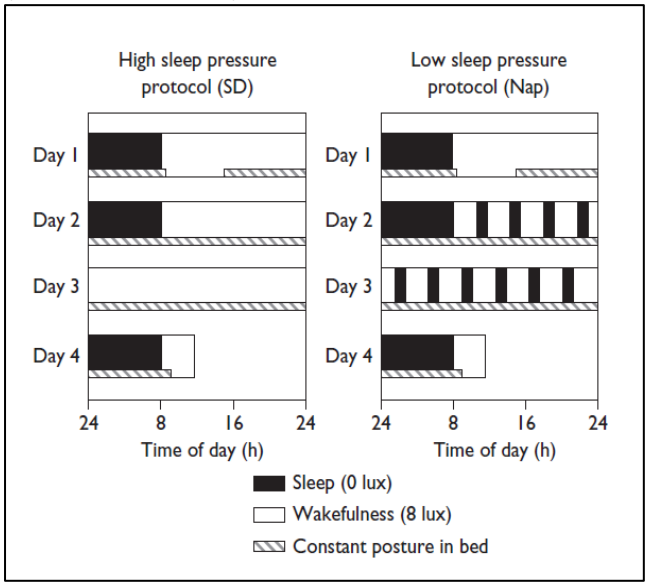
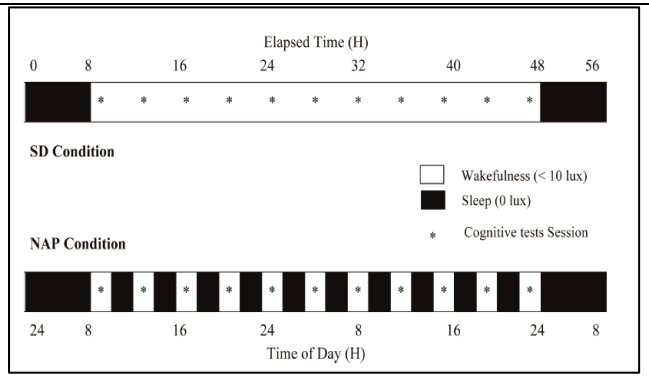
Appendix 1 - Circadian rhythmicity versus sleep homeostasis – experimental protocols – *summary table*

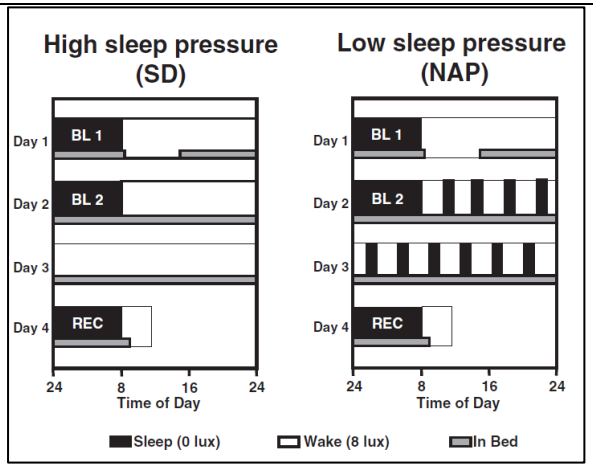
Authors	Participants (N/age/sex/)	Protocol	Main outcome measures	Main results
Graw et al., 2004	n=16 *sex(men)*: 8 age: 25.1±3.4	Two **40-h constant posture protocols** in a balanced crossover design. 1) low sleep pressure conditions (NP) - **150 min** of wakefulness and **75 min** of sleep (10 cycles) 2) high sleep pressure condition (SD) - total sleep deprivation protocol **(40h)** TASK **PVT assessment:** 5-min-long, administered every 225 min starting from 75 min after lights on in the morning (11 trials in total).  	- sustained attention - PVT	Sig. interaction between the *type of protocol* and *time course* was found for the following measures: - **the number of lapses were sig. more frequent in the SD protocol between 23.75h and 35h** (23.75h p=0.05; 27.5h, $p \leq 0.001$; 31.25 p=0.0001; 35h, $p \leq 0.001$); [interaction $F(10,150)=2.7$, $p \leq 0.01$] - **the 90th percentile (slowest RTs) was sig. slower between 23.75h and 38.75** ($p \leq 0.001$) **in SD protocol**; [interaction $F(10,150)=4.5$, $p \leq 0.001$] - **the10th percentile (fastest RTs) revealed no sig. difference between the two protocols** [interaction $F(10,150)=1.7$, p=0.10] - **the difference between 10th-90th percentile of RTs was sig. greater in SD protocol between 20h and 38.78h** (20h p=0.05**;** 23.75h, $p \leq 0.001$**;** 27.5h p=0.0001**;** 31.25h p=0.0001**;** 35, $p \leq 0.001$**;** 38.75h p=0.05); [interaction $F(10,150)=3.3$, $p \leq 0.01$]
Reichert et al., 2017	n=31 *sex(men)*: 14 age: 24.68±3.31	Two **40-hour** conditions; a randomized controlled within-subjects design 1) low sleep pressure conditions (NP) - 10 short sleep-wake cycles, each consisting of **160 min** of wakefulness and **80 min** of a napping opportunity 2) high sleep pressure condition (SD) - **40-hour** total sleep deprivation	- BOLD response -fMRI study - sleep EEG - melatonin assessment - a visual n-back task	**n-back** - the number of hits was sig. worse under SD (37h wake) compared to NP (13h wake) (p=0.01) and NW (2h wake) ($p \leq 0.001$), while performance

		TASK 9 blocks of **the 3-back task** of about 20 min every four hours, starting one hour after waking up from the baseline night **fMRI scans:** Participants were scanned once in the NP and two times in the SD protocol, i.e., 13 hours of wakefulness = normal wake (NW) and 37 hours of wakefulness = under high sleep pressure (SD)	- subjective sleepiness – KSS - melatonin	between the NP and NW conditions did not differ sig. (p=0.78). **fMRI** - differences in the BOLD signal were positively correlated with differences in performance both between NP and SD and NW and SD (NP-SD: cerebellum r=0.44, p=0.01; NW-SD dorsolateral prefrontal cortex r=0.35, p=0.04; all tests of significance: one-sided).
Cajochen et al., 2001	n=10 *sex*$^{(men)}$: 6 age: 27.1±2.3	A **40 h** controlled constant posture protocol; two protocol blocks (4 days each) 1) low sleep pressure protocol (NP) - **150 min** of wakefulness and **75 min** of sleep (10 cycles) 2) high sleep pressure protocol (SD) under constant routine conditions (SD) - total sleep deprivation protocol baseline and recovery nights – 8 h TASKS - KSS every 30 minutes; KDT every hour  	- waking EEG dynamics - core body temperature (CBT) - subjective sleepiness – KSS, KDT	- subjective sleepiness was sig. lower in the NP starting from around midnight until the end of the protocol (p<0.05). - low EEG activity (%) in **the frontal derivation** (1-7 Hz) **increased across the SD protocol**, whereas in the NAP protocol only small changes in the time course were observed (sleep pressure x time: F(10,90) = 4.1; p<0.05). - **the first sig. change in frontal low EEG activity between conditions was at minimum CBT**, with higher values in the high sleep pressure condition. This increase remained sign. for the rest of the protocol (p<0.05). - **frontal EEG beta activity increased** (21±25Hz) across the SD protocol and, to a lesser extent, across the NP - the interaction sleep pressure x time did not yield sign. (F(10, 90).1.5; p=0.20). The time course of CBT did not show any sig. difference between the two conditions (sleep pressure x time: F(31,279)=0.6; p=0.80).

Maire et al., 2018	n=31 *sex(men)*: 14 age: 24.8 ± 3.3	Two study blocks (56h each) in a pseudo-randomized, balanced, crossover order. 1) low sleep pressure protocol (NP) - **160 min** of wakefulness and **80 min** of sleep (10 cycles) 2) high sleep pressure protocol (SD) - total sleep deprivation protocol **(40h)** **-** baseline and recovery nights – **8 h** - FMRI data were acquired at five-time points (sessions), i.e., at 5h, 13h, 21h, 29h, and 37 h into both protocols TASKS KSS; **10-minutes** of PVT in fMRI	- fMRI study - EEG - vigilant attention - PVT - subjective sleepiness - KSS	- **in SD: arousal-promoting thalamic** activation during optimal PVT performance **corresponded to the time course of subjective sleepiness** reaching a peak at night and troughs during the following day. - **task-related cortical activation decreased when sleepiness increased** (higher sleep debt). - no sig. temporal correlations between brain activity during PVT performance and subjective sleepiness were observed in NP.
Sagaspe et al., 2012	*young males* n=14 *sex(men)*: 14 age: 23±2.7 *elderly males* n=14 *sex(men)*: 11 age: 68±1.4	Two **40-h** constant routine protocol 1) low sleep pressure conditions (NP) - **150 min** of wakefulness and **75 min** of sleep (10 cycles) 2) high sleep pressure condition (SD) - total sleep deprivation protocol FOR **40h** TASK - **simple reaction time task**[1] (**10 minutes**) Task was repeated every 3h 45minutes (7h35, 11h20, 15h05, 18h50, 22h35, 2h20, 6h05, 9h50, 13h35, 17h20 and 21h05) - **Go/Nogo task** [2] A total of 576 stimuli divided into **9 task blocks** were shown during the **30 min task**. Task was assessed every 3h45 (8h, 11h45, 15h50, 19h15, 23h, 2h45, 6h30, 10h15, 14h, 17h45 and 21h30) - **VAS sleepiness scale –** assessed before each session	- sustained attention - simple reaction time task - inhibitory motor control - Go/Nogo task - subjective sleepiness - VAS	- in the **SD** condition, **inhibitory motor control** was impaired by extended wakefulness equally in both age groups ($p<0.01$) - sustained attention decreased under sleep deprivation in both groups, and even more in young participants ($p<0.05$) - in the **NP** condition, **age did not impact the time course of inhibitory motor control or sustained attention** - in the SD and NP conditions**, older participants had a less fluctuating RT performance across time of day than young participants** ($p\leq0.001$)

[1] A black square was displayed 100 times on the screen at randomized (2–7 s) intervals over 10 min.
[2] E-prime

				
Birchler-Pedross et al., 2009	n=14 *sex(men)*: 11 age: 68±1.4	two **64-h constant routine protocols** in a balanced crossover design (**3.5-day**) 1) low sleep pressure conditions (NAP) - **150 min** of wakefulness and **75 min** of sleep (10 cycles) 2) high sleep pressure condition (SD) - total sleep deprivation protocol **(40h)** - melatonin and cortisol assessment TASK **- Subjective well-being** - composited score averaged over the 3 items "mood, tension, and physical comfort," each assessed by a 100-mm bipolar VAS, every 30-min; subjects were asked how they feel 'at the moment' - **subjective sleepiness –** assessed by KSS and KSSCL - every 30-min - saliva collections for hormonal assays - every 30-min (during wakefulness)	- VAS: subjective well-being, alertness, hunger, subjective thermal comfort - PANAS - KSS - saliva assays – melatonin and cortisol	- the outcomes show **age-** and/or **gender-related modifications of well-being related to sleep deprivation and circadian phase** - sign. rANOVA for the main factors "**age**' (p =0.01); "**sleep pressure**,"(p=0.09); and "**time of day**"($p \leq 0.001$); **average well-being was sig. lower in older subjects than the young** (56.9 ± 2.2 vs. 65.3 ± 2.1), and **lower during SD than NAP** protocols (59.7 ± 1.9 vs. 62.4 ± 1.6) - the **well-being of older participants was more impaired under SD** conditions than the young (p=0.01) - sig. 2-way interaction **"gender x sleep pressure" under the SD** condition revealed a **decrease in subjective well-being in females but not males** (p=0.003) - **older volunteers were on average more sleepy than the young** ($p \leq 0.04$, Mann-Whitney *U* test)

		 **day 1:** subjects adjusted to the dim light (<8 lux); older participants got heparin injection; **day2,3**: 40h of constant sleep protocol followed by a recovery night (**day 4**); all wake episodes were spent under semi-recumbent CR conditions		
Blatter et al., 2005	n=14 *sex(men)*: 8 age: 65.3±6	Two blocks of 5 days; **40-h constant routine protocols** in a balanced crossover design. 1) low sleep pressure conditions (NP) - **150 min** of wakefulness and **75 min** of sleep (10 cycles) 2) high sleep pressure condition (SD) - total sleep deprivation protocol **(40h)** - adaptation night + baseline night + recovery night TASK - KSS + VAS subjective sleepiness every 30 minutes - maze task was assessed 15-40minutes before each nap and after the recovery night. In the SD protocol, each maze was presented at times corresponding to NAP assessments -PVT	- a planning task – a maze tracing task - (cognitive and motor components were analysed separately) - KSS - VAS – subjective sleepiness - sustained attention – PVT	- **sleep loss-related impairments in planning rely on the difficulty of a given task** - rANOVA with the repeated factors **'difficulty level'** (easy and difficult) and **'session'** (sessions 4–11) **revealed sig. longer performance time of a task for the difficult mazes** (F=20.2, $p \leq 0.001$) - regarding motor execution time of the maze trace **no sig**. differences between the control and experimental group were obtained (F=1.6, p=0.22) - to analyse motor performance in the experimental group exclusively, a rANOVA with the repeated factors **'session'** and **'condition'** was conducted, but **no sig. effects were found** (**regardless of differential sleep pressure condition, motor execution of the maze task was not sig, different between NAP and SD**)

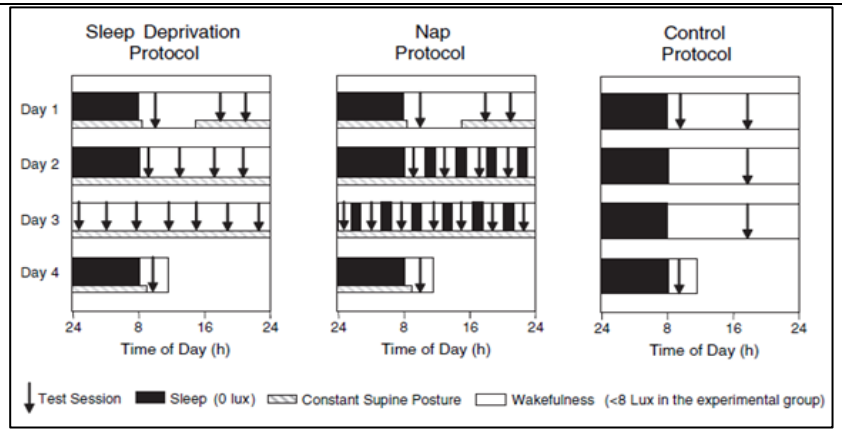
				- rANOVA for **the difficult mazes**, where the **'session' x 'condition'** interaction was significant (F= 20.6; p=0.05) - none of the subjective sleepiness scales (KSS, VAS) nor any of the PVT performance measures showed predictive power in relation to planning performance

Table 1. Experimental protocols investigating circadian rhythmicity versus sleep homeostasis. All selected studies included low sleep pressure conditions, i.e., Nap Protocol (NP) and high sleep pressure conditions, i.e., Sleep Deprivation protocol (SD). *abbreviations: BOLD* – Blood-oxygen-level-dependent imaging; *EEG* – Electroencephalography; *KSD* – Karolinska Sleep Dairy; *KSS* – Karolinska Sleepiness Scale; *PANAS* - Positive and Negative Affect Schedule; *PVT* – Psychomotor Vigilance Task; *RT* – Reaction time; *sig*. – significant; *VAS* - Visual Analogue Scale

Appendix 2 - Impact of APOE polymorphism on sleep in cognitively intact adults – *summary table*

	Authors	Participants (N/age/sex/)	APOE genotype (N)	Main outcome measures	Main findings
subjective sleep measures	Asada et al., 2000	patients with AD suspicion n=337; *sex(men)*: 144; age: 73±9 n=260 controls (non-demented spouses of the AD patients); *sex(men):* 94; *age:* 69±9	ε4 allele present (ε2/ε4, ε3/ε4, ε4/ ε4) = 240 ε4 allele absent (ε2/ε2, ε2/ε3, ε3/ ε3) = 357	- habitual napping habits over the last 5 and 10 years before the onset of AD (retrospective assessment)	**Duration of taken naps interacted with APOE-ε4 genotype.** Long naps (i.e., <60 min) were related to a higher AD risk across ε4 carriers (OR=4.37) but not in noncarriers (OR=1.71). Short naps (i.e., <30 min) were associated with reduced risk of AD incident in ε4 carriers (OR=0.08, CI=0.02 - 0.32) and Ɛ4 non-carriers (OR=0.20, CI= 0.09 - 0.43).
	Spira et al., 2017	n=1264 cognitively normal adults; *sex(men):* 655*; age:* 57.5±16.1	elevated risk (ε2/ε4, ε3/ε4, ε4/ε4) = 352 neutral for AD risk (ε3/ε3) = 750 lower risk: (ε2/ε2, ε2/ε3) = 162	- self-reported sleep duration, i.e., ≥9, 7 or 8 or ≤6 hours, difficulty with falling/maintaining sleep, and napping habits	**APOE genotype modulates vulnerability to sleep disturbance.** ε4/ε4 carriers had a 38% greater odds of reporting **a shorter sleep duration** compared to all noncarriers (OR=1.41) and ε3/ε3 group (OR=1.43). ε2/ε2 or ε2/ε3 carriers had a 38% lower odds of napping in comparison to ε3/ε3 carriers (OR=0.64). In a subset of participants aged ≥50 years, ε4 carriers had 50% higher odds of reporting difficulties in falling/staying asleep compared to noncarriers (OR=1.49).
	Tsapanou et al., 2019	non-demented older adults n=1944, *sex(men):* 610*; age:* 79±7	ε4 carriers (ε2/ε4 were excluded) = 484 non-ε4 carriers = 1460	- Sleep Scale from the RAND Medical Outcomes Study questionnaire	**None of the sleep variables such as sleep disturbance, sleep adequacy and daytime sleepiness was shown to be associated with APOE-ε4.** APOE-ε4 is associated with decreased occurrence of snoring (*p*=0.01) and sleep apnoea (*p*=0.04).
	Camargos, E., et al., 2019	non-demented subjects n = 163; *sex(men):* 24 *; age* = 75.3 ± 7.0	ε2 carriers (ε2ε2, ε2ε3) = 14 ε3 carriers (ε3ε3) = 121 ε4 carriers (ε3ε4, ε4ε4) = 28	- PSQI - ESS	**APOE status was significantly correlated with PSQI global score (*p*=0.04), with a greater frequency of the poor sleep quality among ε2 carriers.** No relationship was found between the APOE and ESS (*p*=0.85).

objective sleep measures	Ju et al., 2013	cognitively normal participants n = 142; *sex(men): 58; age* = 65.6 ±8.2	ε4 carriers = 52	- actigraphy (14 days) - CSF Aβ42 levels	**Carriership of the ε4 allele was associated with an increased risk of amyloid deposition in the preclinical stage of AD and was associated with decreased SE.** High CSF Aβ42 was significantly correlated with poorer sleep quality (lower mean SE) compared to normal levels of Aβ42 (80.4% versus 83.7%, t-test *p*=0.01). After correction for age, gender, and APOE-ε4 allele carriership status using ANCOVA, the two groups maintained a significant difference in sleep efficiency (*p*=0.04).
	Drogos et al., 2016	non-demented subjects n=35; *sex(men):* 14; *age:* 65 ± 5	APOE-ε4+ status (ε2/ ε4, ε3/ ε4, ε4/ ε4) = 8; APOE-ε4- status (ε2/ε2, ε2/ ε3, ε3/ ε3) = 27	- at-home PSG - actigraphy - PSQI	**APOE-ε4 was shown to lead to significantly worse objective sleep quality in absence of subjective sleep complaints.** ε4 carriers had shorter sleep duration (*p*=0.01), longer WASO (*p*=0.01) and lower SE (*p*=0.02) compared to noncarriers. ε4+ individuals had a lower percentage of TST in N2 (*p*=0.03), and more time spent in REM sleep (*p* =0.01) compared to ε4 non-carriers. APOE-ε4 status was not significantly associated with self-reported sleep measures.
	Kahya, M., et al., 2017	cognitively normal older adults without self-reported sleep apnoea n=36 s; *sex(men):* 11; *age* = 72±5.7	ε4 carriers (ε3/ε4, ε4/ε4) = 9; ε4 noncarriers (ε2/ε3, ε3/ε3) = 27	- actigraphy and a sleep log (7 days) - PSQI - ESS	**The APOE-ε4 carriers had a higher number of awakenings compared to the non-carriers (*p*=0.02).** There were no significant group differences in sleep latency, SE, TST, WASO, ESS and PSQI global score, besides the PSQI subcomponent of daily disturbances was significantly higher in APOE-ε4 carriers (*p*=0.03).
	Tranah et al., 2018	non-demented subjects n = 1747*; sex(men):* 1747; *age:*76	zero ε4 allele = 1747; one ε4 allele= 515; two ε4 allele = 40	- in-home, single night PSG - Modified Mini-Mental State Exam (3MS)	**APOE-ε4 was associated with increased stage N3 duration. APOE-ε4 status is associated with lower cognitive function scores.** Total time in N3 sleep was significantly higher (*p*≤0.0001) in ε4 homozygotes (62±5.2 min) compared to ε3/ε4 (43±1.5 min) and ε4 non-carriers (40±0.8 min).
	Hwang et al., 2018	cognitively normal adults n=133; *sex(men)*:62*;* age: 68.05±7.68	APOE- ε4 noncarrier = 108 APOE- ε4 carrier = 25	- actigraphy and sleep dairy (8 days) - PET-MRI to evaluate cerebral Aβ deposition	**APOE-ε4 allele seems to moderate the relationship between the sleep-wake cycle and Aβ accumulation in cognitively normal older adults.** There was a positive interaction between APOE-ε4 status with sleep latency (*p*=0.01), mesor (*p* =0.02) acrophase (p=0.01) and Aβ positivity. In APOE-ε4 noncarriers, advanced acrophase was associated with increased Aβ positivity. In APOE-ε4 carriers delayed acrophase was associated with Aβ

					positivity (p=0.05). In APOE-ε4 noncarriers shorter SL (p=0.002), higher mesor (p=0.01), and advanced acrophase (p=.013) were associated with Aβ positivity. After Bonferroni correction, shorter SL and higher mesor were still significantly associated with Aβ positivity in APOE-ε4 noncarriers.
	Muto et al., 2020	cognitively normal men without sleep disorders n= 363; age: 22.1 y ± 2.7	ε4 carriers = 100 ε4 non-carriers = 263	- 7-day protocol (where PSG data from baseline night, extension nights (a 12h sleep opportunity), recovery sleep after total sleep deprivation (40h) were included in the analysis) - ESS - Polygenic Risk Scores (PRS) for AD	**There was no difference in slow-wave energy (SWE) (p=0.84) and daytime sleepiness (p=0.94) between APOE-ε4 carriers and non-carriers.**
	López-García et al., 2021	non-demented adults n= 127; age: 65.5 y ± 6.3; *sex(men)*: 38	ε4 carriers (carriers of ≥1 ε4 allele) = 39 ε4 noncarriers (non-carriers of ε4 allele) = 88	- 7-days of actigraphy - AD CSF biomarkers	**TST is an early marker of tau pathology and APOE genotype modulates this relationship and is driven by ε4 allele carriers.** The interaction between TST and APOE-ε4 status significantly predicted t-tau levels (β=0.78; p=0.02). ε4 allele carriers had lower TSTS compared to non-carriers (422.3±81.6 vs. 451.57±67.13 min, respectively; p=0.04). No associations were found between memory performance and TST.
longitudinal studies	Lim et al., 2013	older adults without dementia n= 698; *sex(men):* 161; *age:* 81.7	ε4 carriers (ε3/ε4, ε2/ε4, ε4/ε4) = 149 ε4 noncarriers (ε2/ε2, ε2/ε3, ε3/ε3) = 549	- 10 days of actigraphy - longitudinal study with 6 years of follow-up to determine incident dementia	**Greater sleep consolidation reduced the effect of the ε4 allele when considering the annual rate of cognitive decline** (p≤0.001) and neurofibrillary tangles density (p=0.02).
	Sapira et al., 2016	n=122 cognitively normal adults at baseline; *sex(men):* 68; *age:* 66.6±8	ε4 carriers = 34 ε4 noncarriers = 88	- a question about how many hours of sleep the participant obtained on average over the past month (responses range: 1-12h)	**Self-reported sleep duration was shown to be influenced by the presence of the ε4 allele**, where shorter sleep (<7h) was more frequent among ε4 allele carriers. APOE-ε4 allele carriers reporting <7h demonstrated higher rates of thinning in the inferior frontal gyrus of the left hemisphere, while those reporting >7 h had higher rates of thinning in the superior frontal sulcus of the left hemisphere when compared to those reporting 7h sleep

				- longitudinal study with 8 years of follow-up assessing cortical thinning on MRI	(p<0.05 for all). Further, ε4 carriers sleeping >7 h had higher rates of thinning in the temporal pole of the right hemisphere, in comparison to individuals reporting sleep duration <7 h (cluster p<0.05).
	Burke et al., 2016	cognitively asymptomatic participants n=8.762 (followed up); *sex*$^{(men)}$: 2.906; *age:*71± 10.89	contains ε2 (ε2ε2, ε2ε3, ε2ε4) = 1.235 APOE-ε3ε3 = 4.855 APOE-ε3ε4 = 2.064 APOE-ε4ε4 = 244	- depression (self-report depression in the last two years, other episodes of depression, clinician-confirmed depression) - sleep disturbances using the Neuropsychiatric Inventory Questionnaire (NPI-Q) - longitudinal study (days until that AD diagnosis occurred ranged from 286 to 3,229 days)	**APOE-ε4 allele carriers who reported sleep disturbance were at almost 7 times greater risk of developing AD than APOE-ε3ε3 (HR=6.79 [2.38-19.37]).**
	Lysen et al., 2020	non-demented adults = 1322; *sex*$^{(men)}$:621; age: 66±8	≥1 ε4 allele = 346 no ε4-alleles = 905	- actigraphy and daily sleep dairy (median=6 days) - longitudinal study with 11.2 years of follow-up to determine incident dementia	Stratifying by APOE-ε4 indicated associations between sleep outcomes (i.e., sleep, bedtime, and 24-hour activity rhythm parameters) and increased risk of dementia in ε4 negative individuals but **no sleep-by-APOE-ε4 interaction term survived multiple testing.**

Table 1. Summary of the peer reviewed journal articles on sleep and circadian rhythmicity involving APOE genotyping. Note that only studies involving cognitively intact participants were included.

Study protocol – screening session

Subjects ID: Date:

Check the box after finishing each task to keep track of the protocol

- ☐ PRESENTATION
- ☐ INFORMED CONSENT

1. PRIORITY QUESTIONNAIRES

starting time: _____

- ☐ Demographics Questionnaire
- ☐ General Medical Questionnaire
- ☐ the Patient Health Questionnaire (PHQ-9)
- ☐ Epworth Sleepiness Scale (ESS)
- ☐ Pittsburgh Sleep Quality Index (PSQI)
- ☐ the Insomnia Severity Index
- ☐ Morning- eveningness questionnaire
- ☐ the Munich Chronotype Questionnaire
- ☐ the Generalized Anxiety Disorder Questionnaire (GAD-7)
- ☐ Cognitive Change Index (CCI)

finishing time: _____

2. NON - PRIORITY QUESTIONNAIRES – the following questionnaires should be administered only if time allows, otherwise, give the subject the pre-paid envelop with unfinished questionnaires (total number of pages of the questionnaires cannot exceed 16)

starting time: _____

- ☐ Edinburgh Handedness Inventory
- ☐ the Dimensional Apathy Scale (DAS)
- ☐ Empathy Quotient (EQ-40) – the Cambridge Behavioural Scale
- ☐ the Barratt Impulsivity Scale (BIS-11)
- ☐ Big Five Inventory
- ☐ Positive & Negative Affect Scale (PANAS)
- ☐ SF36 Health Survey
- ☐ Dutch Eating Behaviour Questionnaire (DEBQ)
- ☐ the Mannheim Dream Questionnaire (MADRE)

finishing time: _____

☐ 10 minutes BREAK

Before giving subjects the refreshment, take buccal swabs

☐ TAKE BUCCAL SWABS – 30 sec of rubbing;

☐ MAKE A COPY OF THE INFORMED CONSENT

3. COGNITIVE ASSESSMENT

starting time: _____

☐ Is the subject left- or right- handed? (circle correct) left right

QUESTIONNAIRES

☐ Karolinska Sleep Diary (KSD)

Assessment of sleepiness before competition of the tasks

☐ Karolinska Sleepiness Scale (KSS)

PAPER – based tasks

☐ Mini-Addenbrooke's Cognitive Examination (M-ACE)

☐ the Rey-Osterreth Complex Figure Test – copying

time taken to complete: _____

☐ Symbol Digit Modalities Test

☐ the Rey-Osterreth Complex Figure Test – immediate recall

time taken to complete: _____

☐ Hopkins verbal learning – encoding

☐ Trail Making Test A and B

time taken to complete (Part A): _____

time taken to complete (Part B): _____

TABLET - based tasks

☐ Supermarket task – trials from 1 to 7

DELAYED RECALLS – Hopkins – 20-25 minutes after the last encoding trial; Rey – 30 minutes after the copy trial

☐ Hopkins verbal learning – delayed recall

☐ Hopkins verbal learning – recognition part

☐ The Rey-Osterreth Complex Figure Test – delayed recall

time taken to complete: _____

☐ The Rey-Osterreth Complex Figure Test – recognition part

Assessment of sleepiness after competition of the tasks

☐ Karolinska Sleepiness Scale (KSS)

finishing time: _____

BEFORE THE SUBJECT LEAVES

- ☐ Give the subject a copy of the informed consent
- ☐ Give the subject the *thank you* letter
- ☐ Give the subject the expense form and the pre-paid envelope if needed

AFTER THE SUBJECT LEAVES

- ☐ Take a copy of the filled out expense form and send it to the finances

COMMENTS

Appendix 4 – qPCR plate preparations' protocol

Preparation before starting real-time PCR APOE SNP genotyping assay

1. Dilute SNP Genotyping Assays.
2. Vortex, then centrifuge the mixture.
3. Dilute 40x or 80x SNP Genotyping Assay to a 20x working stock with 1x TE buffer. For example dilute 25 µl 40x solution with 25 µl 1x TE buffer.
4. Aliquot the 20x working stock into tubes containing 50 µl (enough for one 96-well plate). [0.5 µl SNP Assay used per reaction, so in total 100 reactions].
5. Cover the tubes with aluminium foil to protect them from light and store at –20 °C (range –15 to –25 °C).

Note: SNP Genotyping assay should preferably not have more than 3 freeze-thaw cycles, and definitely not more than 10 freeze-thaw cycles. SNP genotyping assays are stable for 3-5 years when properly stored and repeated freeze-thaw cycles are avoided.

9.3.2 Real-time PCR APOE SNP genotyping assay

The APOE genotype will be determined using two RT-PCR SNP Genotyping Assays, which determine the 112 T/C (rs429358) APOE4 polymorphism and 158 C/T (rs7412) APOE2 polymorphism.

Preparation of the PCR plate (BCRE lab):

1. Spray the work bench with hot soapy water. Clean pipettes with ethanol.

2. Label 1.5ml sterile Eppendorf tubes, 1 per sample. Label with volunteer ID and date.

   Label 2ml sterile Eppendorf tubes, 1 with 'MM 112' the other with 'MM 158'.

   Label a 2ml sterile Eppendorf tubes with 'Water' (this will be used for the NTCs).

   Write up a 96 well reaction plate set-up map to show where each sample will be placed in the reaction plate (M:\MED\Groups\DH Lab\AMM Lab\CANN Study\APOE analysis\CANN genotyping) Done in excel file.

3. Defrost the Master Mix, primer/probes (i.e., the genotyping assays) and DNA samples at room temperature. Place on ice. The primer/probes must remain in their foil wrap to protect from light.
4. Pipette 5µl of DNA sample into the 1.5ml labelled Eppendorf tube. Then pipette the calculated amount of molecular grade water (RNA/ DNA free). Repeat for each DNA sample. Place the rack of diluted samples on ice.
5. Pipette 1ml of molecular grade water into the Eppendorf tube labelled 'water'. This will be used for the NTC's. Put this in the rack with the other samples.

6. Prepare the stock mixture of Master Mix and primer/probes in a sterile Eppendorf as indicated in the table below. You will make a stock mixture for 158 and another stock mixture for 112. To ensure you make enough stock, multiply the volumes in the table below by number of reactions (i.e. samples plus positive controls plus no template controls (NTCs)) plus 3 (to compensate for reagent transfer loss). (Recommended to have 2 positive controls when only running 1-20 ish samples, like we will be for CANN). See example of how to calculate the amount of stock to make (p18)

Gently swirl the bottle of TaqMan Genotyping Master Mix (2x) before use, to ensure it is well mixed. Pipette the required volumes (master mix, primer/probe and molecular grade water) in a pre-labelled sterile Eppendorf (labelled 'MM112' and 'MM158'), cap the tube and invert several times to mix and perform a short spin in the bench centrifuge to eliminate any air bubbles from the reaction mix. Wrap in foil and place on ice. (Any remaining stock can be frozen and stored for up to a year and pooled if necessary with freshly prepared stock. Only freeze thaw once).

Component	7500 Fast RT-PCR (10 µL rxn volume)
TaqMan Genotyping Master Mix (2X)	5.0 µL
Primer/probe (20X working stock of SNP Genotyping Assay)	0.5 µL
RNase and DNase free water	2.5 µL
Total Volume per Well	8 µL

7. Place the 96-well reaction plate into a solid clean black plate holder (the ones with holes that go right the way through-like a stand rather than a tray). With a marker pen label the reaction plate as necessary. For CANN we will likely run a plate with 112 mastermix on one half and 158 mastermix on the other half as we will never have too many samples in any one run. So label which side of the plate is for which assay and draw a little divide line to show where the plate splits in the middle (this is shown on the plate set-up map).
   Pipette 8µL of prepared Mastermix mix into each well that is required (for this you can use the same pipette tip to do all the 112's, then change the tip and do the 158's). Avoid touching the outside of the reaction plate but its ok if you touch the inside of the wells with the tip. Check all the necessary wells contain the mastermix before continuing.

   Pipette 2µL of the DNA sample (containing 1-20 ng purified genomic DNA) into the indicated well (refer to the 96 well reaction plate set-up map you made earlier) and 2µL of RNase/DNase free water for the NTC's. Change the pipette tip between each sample, even the NTC'S. It is recommended to have 3 NTCs on each SNP genotyping reaction plate. Important: Be sure that no well to well cross-contamination occurs during pipetting.

8. Inspect all wells for uniformity of volume, and make note of any wells which do not appear to contain the proper volume.

9. Place a plastic disposable cover (onto the plate) and use paddle to ensure that it is firmly pressed down. Vortex the plate to mix the wells and centrifuge the plate briefly at 1,000 rpm to spin down the contents.
10. Keep the reaction plate wrapped in foil and in a fridge if you are make up the plate a few hours before loading the plate in the 7500 Fast Real-Time PCR system. Placing the plate on ice is not necessary as Rose said this will just make it wet.

Appendix 5 – Exemplary qPCR plate set-up

10th PCR plate – 03.12.2020

112

158

	1	2	3	4	5	6	7	8	9	10	11	12
A	S025 TT +Control	133 B	137 B	141 B	125 A		COB349 TT +Control	133 B	137 B	141 B	125 A	
B	S112 CC +control	134 A	138 A	23 A	126 A		S025 CC +control	134 A	138 A	23 A	126 A	
C	S013 CT +control	134 B	138 B	23 B	34 A (5s:5w)		S013 CT +control	134 B	138 B	23 B	34 A (5s:5w)	
D	118 A	135A	139 A	24 A	41 A		118 A	135A	139 A	24 A	41 A	
E	118 B	135 B (5s:5w)	139 B	24 B	53 A		118 B	135 B (5s:5w)	139 B	24 B	53 A	
F	132 A	136 A (5s:5w)	140 A	64 B		NTC	132 A	136 A (5s:5w)	140 A	64 B		NTC
G	132 B	136 B (5s:5w)	140 B	65 A		NTC	132 B	136 B (5s:5w)	140 B	65 A		NTC
H	133 A	137 A	141 A	39 A		NTC	133 A	137 A	141 A	39 A		NTC

- Fill all NTC (negative template controls) with pure water
- C encodes for Allele1 and T encodes for Allele 2
- Empty cells are not filled with liquid
- *all samples with asterisk need to be diluted

* Example – indicating that dilution is needed:
76_A
dilution (5s:5w)

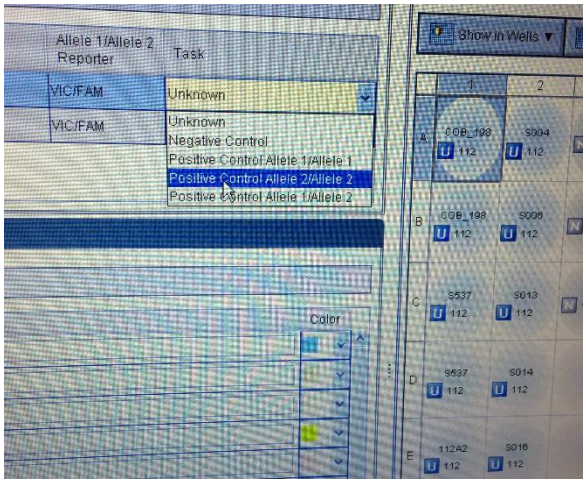
Appendix 6 – qPCR machine set-up

Setting qPCR machine

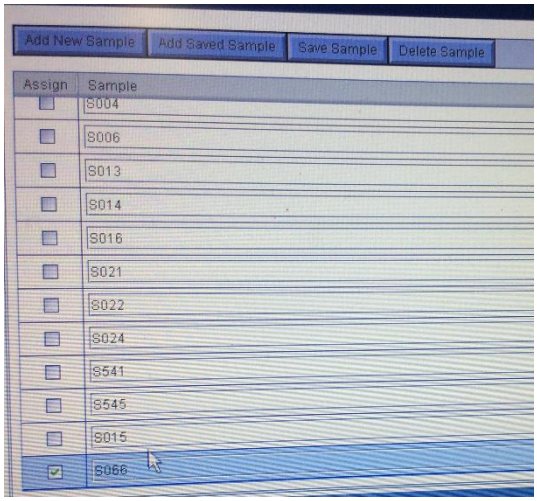
1. Set a new project with two SNP assays, i.e., side 112 and side 158.
2. Click on each of the positive controls and assign to the correct allele, this can be selected from the dropdown under 'task. (see below). There are three positive control for 112 and three for 158.

SAMPLES	GENOTYPE	112	158
S025	e3/e3	2/2 (TT)	1/1 (CC)
S013	e2/e4	1/2 (CT)	1/2 (CT)
COB349	e2/e2	2/2 (TT)	2/2 (TT)
S112	e4/e4	1/1 (CC)	1/1 (CC)

3. Do the same for the negative controls

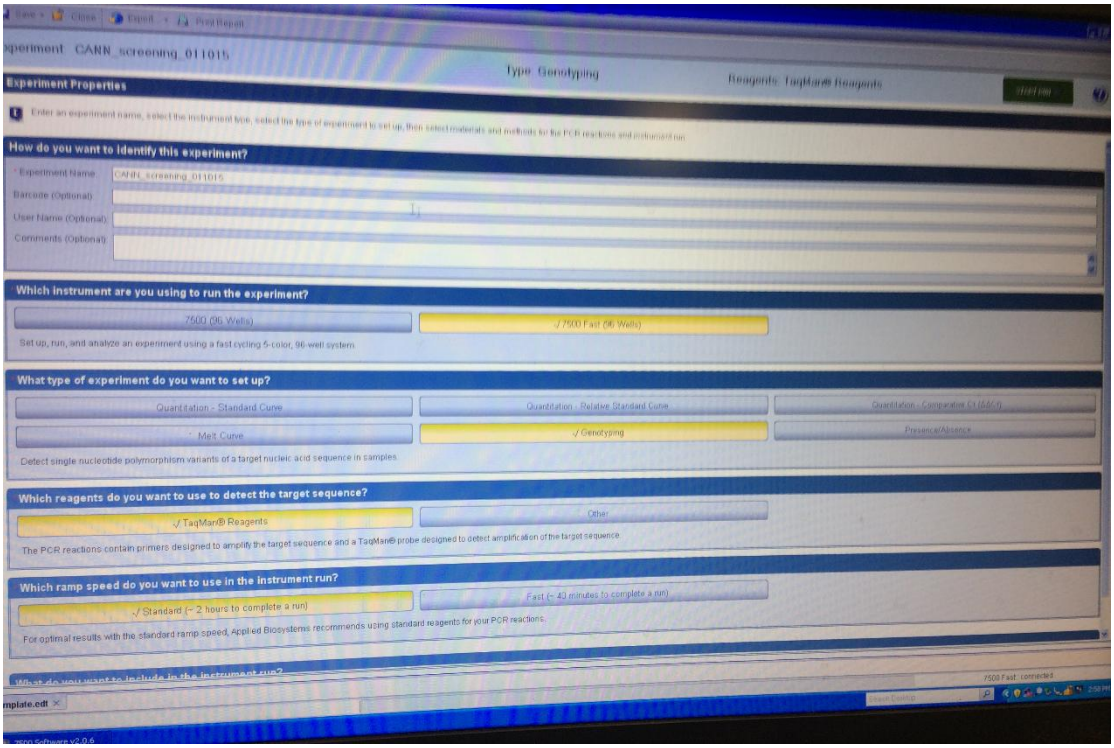


4. Now one by one, assign ID's to the wells. Do this by clicking on the relevant well and then either click the tick box to assign and existing ID number or click 'add new sample' and the create a new ID label, then tick the box next to the new ID (see below). Make sure the right well now has the correct ID label. Do this for all your samples and the positive controls



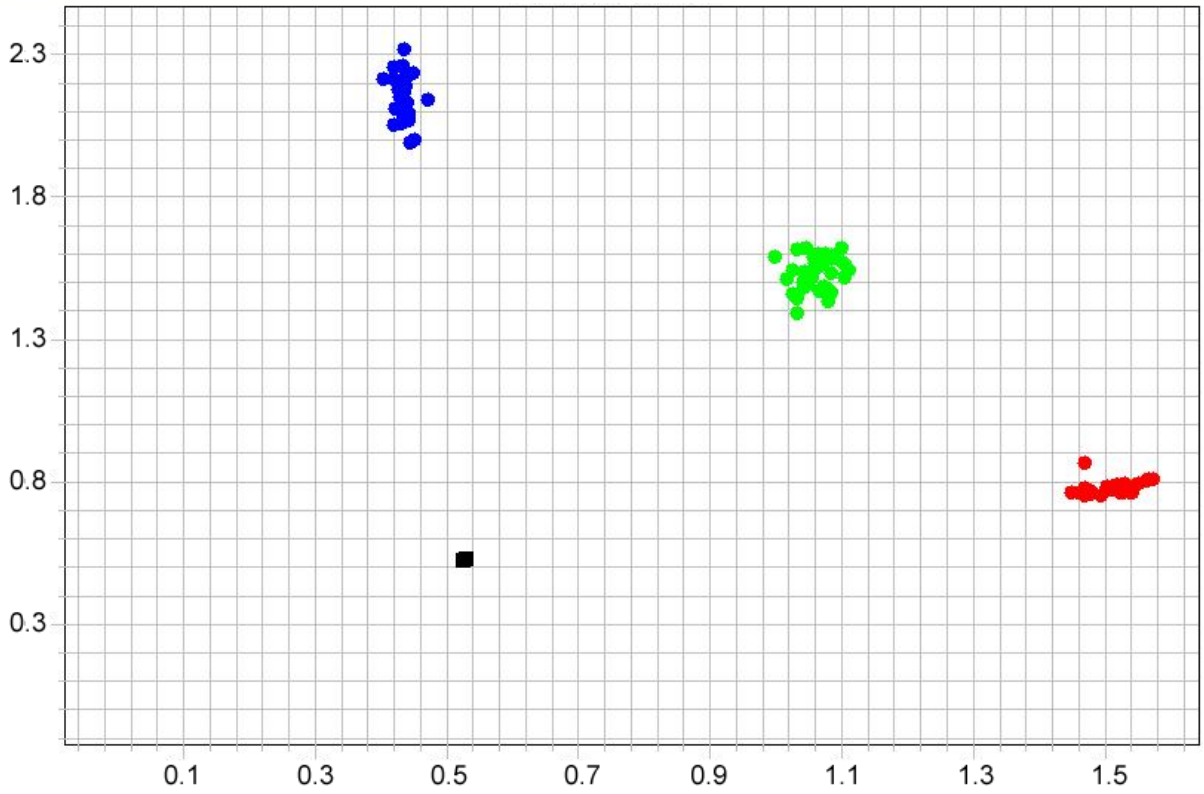
Your set up will now look something like this:

5. Once you are happy everything is labelled correctly click the 'experiment properties' tab on the left of the screen and check the set up looks like this:



6. Then click the 'run method' tab and check the amount per well is set to **10 µl**
7. Then click '**save**' and specify the name and date to suit
8. Put sample plate in the machine **with the notch in the back left corner**

9. Pres ‘run’ and check it starts to run by waiting for the time to start counting down. This can take a couple of minutes. The run will then take 90 minutes.
10. Once the run has finished (be sure it has before you press anything!), highlight all the wells and then save as and export.



Allelic Discrimination plot

Blue is homozygote allele 2 (TT)

Green is heterozygote allele 2 (CT)

Red homozygote allele 1 (CC)

Black: no template control

University of East Anglia

A study on genes, sleep and memory

Early sleep and circadian markers of Alzheimer's disease: The impact of APOE-ε4 on circadian rhythm and sleep-wake homeostasis in humans

WEEK ONE DIARY

If you have any queries please contact

Dr Alpar Lazar (Principal Investigator)

Phone: 01603 597539

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A study on genes, sleep and memory: Version 2.0, 31/08/2018
FMH Research Ethics Committee Reference: 2017/18 - 135

A study on genes, sleep and memory Particpant ID: APO

A study on genes, sleep and memory

Instructions:

Wearing the watch

- You should wear the actiwatch continuously on your non-dominant wrist during the 2 weeks.
- You can take it off occasionally for a short time but please do not forget to put it back afterwards. The actiwatch is waterproof.
- The actiwatch should be fastened as you would a normal wrist watch. You can adjust the strap to ensure this is comfortable on your wrist.

Precautions

- Actiwatches are not designed to be worn on irritated or damaged skin. In the unlikely event that you experience any discomfort or irritation please try to wear the actiwatch on you other wrist. Ideally the actiwatch should stay on your non-dominate wrist.
- Nevertheless, if you do need to change it please make a note in your sleep diary. If you continue to experience discomfort please notify the study team.
- As with similar devices you should also ensure that the actiwatch is kept out of reach of children as it contains a coin cell battery that can pose a potential swallowing hazard if the casing were to be opened. You are not required to open the actiwatch while it is in your possession.

When you return for your second MRI scan, a member of the study team will collect the watch and the diary from you, so please make sure you bring them along with you.

Day 1

Morning questionnaire

Date:……. Time:……

The following sleep questions should be completed in the morning after you have woken up. Please fill in the date and time above. You should try to complete these questions at the time rather than providing answers retrospectively if possible. *Please indicate whether the time is AM or PM.*

What time did you go to bed?		**AM/PM**
What time did you try starting to sleep at?		**AM/PM**
How long did it take you to fall asleep? (min)		
How many times did you wake up?		
How long were you awake for? (min) Please estimate the time and duration of each night awakening		
What time did you wake up?		**AM/PM**
What time did you get up?		**AM/PM**
Did your alarm clock wake you up?		
Did you do any strenuous activity during the last 24 hours (if yes, please specify)		

Day 1 - Morning

How would you rate your quality of sleep?

1 2 3 4 5 6 7 8 9
Best sleep ever. Worst sleep ever

How difficult did you find to wake up/get up?

1 2 3 4 5 6 7 8 9
Very easy Quite hard

Please indicate your level of sleepiness for the previous 5 minutes using the scale below (circle appropriate number)

Extremely alert	1
	2
Alert	3
	4
Neither sleepy nor alert	5
	6
Sleepy, but not fighting sleep	7
	8
Extremely sleepy; it is an effort to stay awake	9

Do you recall your dream/s from your last sleep episode/nap? (circle)

Yes **No**

If yes, how intense were your dreams emotionally (please circle the number that applies)?

1	**2**	**3**	**4**	**5**
Not at all intense	Not that intense	Somewhat intense	Quite intense	Very intense

What was the emotional tone of your dreams on average (please circle the number that applies)?

1	**2**	**3**	**4**	**5**
Very negative	Somewhat negative	Neutral	Somewhat positive	Very positive

Did you experience a so-called lucid dream/s (see definition)?
Definition: In a lucid dream, one is aware that one is dreaming during the dream. Thus it is possible to wake up deliberately, or to influence the action of the dream actively, or to observe the course of the dream passively.

Yes **No**

If yes and you feel comfortable providing details please provide these below:

……
……
……
……
……
……

A study on genes, sleep and memory Particpant ID: APO

Day 1

Evening questionnaire

Date:…….. Time:……

You should complete the following questions at the end of each day. Please fill in the date and time above.

Over the last 24 hours, how often have you been bothered by the following problems? *Tick the most appropriate box below for the following statements.*

	Not at all	Several times	More than half the time	Nearly all the time
Feeling nervous, anxious or on edge				
Not being able to stop or control worrying				
Little interest or pleasure in doing things				
Feeling down, depressed or hopeless				

The following questions will ask you about the time you spent being physically active today.

Please answer each question even if you do not consider yourself to be an active person.

To describe the intensity of the physical activity, two terms (Moderate and Vigorous) are used:

Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.

We understand that you cannot remember exactly all the activities and the actual time when those happened but please try to complete the questionnaire to the best of you knowledge by estimating the duration of those activities.

The first question is about the time you spent sitting during the day. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the day, how much time did you spend sitting in total?

____ hours ___ minutes (total)

When: □ morning, □ early afternoon, □ late afternoon , □ evening

(please tick at least one)

Think about the time you spent walking today. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

During the day how many times did you walk for at least 10 minutes?

_____ times

How much time did you spend walking today in total?

_____hours ___ minutes

When: □ morning, □ early afternoon, □ late afternoon , □ evening

During the day how many times did you do moderate physical activities like gardening, cleaning, bicycling at a regular pace, swimming or other fitness activities.

Think only about those physical activities that you did for at least 10 minutes at a time. Do not include walking.

_____ times

How much time did you spend doing moderate physical activities today?

____hours ___ minutes (total)

When: □ morning, □ early afternoon, □ late afternoon , □ evening

During the day, how many times did you do vigorous physical activities like heavy lifting, heavier garden or construction work, chopping woods, aerobics, jogging/running or fast bicycling?

Think only about those physical activities that you did for at least 10 minutes at a time.

_____ times

How much time did you spend doing vigorous physical activities today?

____hours ___ minutes (total)

When: □ morning, □ early afternoon, □ late afternoon , □ evening

What is the average time you have spent outdoors exposed to direct sunlight?

____ hours ___ minutes (total)

When: □ morning, □ early afternoon, □ late afternoon , □ evening

How many caffeinated beverages have you had today? (e.g. tea/ coffee/coke)

____ cups

When: □ morning, □ early afternoon, □ late afternoon , □ evening

How many alcohol units have you had today? (One unit is half a pint of beer / one glass of wine/ one measure of spirits)

____ units

When: □ morning, □ early afternoon, □ late afternoon , □ evening

How many times did you have a meal or a snack today

a. Meal: _____

b. Snack: _____

When was the first and the last time you had a meal today and what did it include?

First: _____ □ Sweet, □ Fatty, □ Savoury

Last: _____ □ Sweet, □ Fatty, □ Savoury

Did you take a nap today? (Circle)

Yes No

If yes how long was the duration of your nap/s in total? ……..

Please indicate your level of sleepiness for the previous 5 minutes using the scale below (circle appropriate number). **Please complete this just before going to bed.**

Extremely alert	**1**
	2
Alert	**3**
	4
Neither sleepy nor alert	**5**
	6
Sleepy, but not fighting sleep	**7**
	8
Extremely sleepy; it is an effort to stay awake	**9**

END OF DAY 1

Mental effort feedback – Pilot – Sea Hero Quest

The participants (n=8) were asked to fill out the subjective assessment after playing each level.

How cognitively demanding was for you completing the level ___?

1	2	3	4	5
not at all				very

Results

Descriptive Statistics

levels	3	6	7	8	11	12	13	16	17	18	21	22	23
M	1.000	1.000	1.222	2.182	2.000	2.167	2.800	2.600	2.333	2.400	3.583	2.750	2.500
SD	0.000	0.000	0.4410	1.079	0.6667	1.169	1.095	1.174	0.7071	0.8433	1.201	0.9653	1.069

Table 1. Mean (*M*) and standard deviation (*SD*) following results of mental effort scale. Levels 3, 6, 7, 8, 11, 12, 17 are consider as "easy levels" and levels 13, 16, 18, 21, 22, 23 are consider as "difficult levels".

Test of Normality (Shapiro-Wilk)

		W	*p*
easy_score	- difficult_score	0.925	0.002

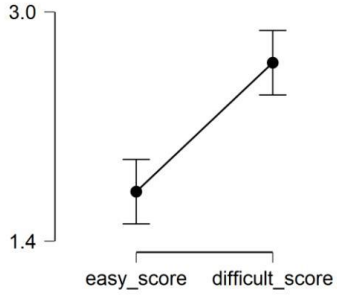
Note. Significant results suggest a deviation from normality.

Wilcoxon signed-rank test

		W	*p*	Rank-Biserial Correlation
easy_score	- difficult_score	133.0	< .001	-0.833

Descriptives

	N	Mean	SD	SE
easy_score	56	1.745	0.938	0.125
difficult_score	56	2.645	1.003	0.134



Lab form 1

Subject code: _____ Date: _____ Time: _____ (To be completed by study team)

Over the last 4 hours, how often have you been bothered by the following problems? *Circle the most appropriate number below for the following statements.*

	Not at all	Several times	More than half the time	Nearly all the time
1. Feeling nervous, anxious or on edge	**0**	**1**	**2**	**3**
2. Not being able to stop or control worrying	**0**	**1**	**2**	**3**

Choose the answer on how you have felt, behaved or thought, based on the rate of occurrence in the last 4 hours: *(Tick the statement that applies)*

1.I am able to focus on a task until it is finished

- ☐ Almost always
- ☐ Often
- ☐ Occasionally
- ☐ Hardly Ever

2. I feel indifferent to what is going on around me
   - ☐ Almost always
   - ☐ Often
   - ☐ Occasionally
   - ☐ Hardly Ever

3. I set goals for myself
   - ☐ Almost always
   - ☐ Often
   - ☐ Occasionally
   - ☐ Hardly Ever

Over the last 4 hours, how often have you been bothered by any of the following problems? *Circle the most appropriate number below for the following statements.*

	Not at all	Several times	More than half the time	Nearly all the time
1. Little interest or pleasure in doing things	**0**	**1**	**2**	**3**
2. Feeling down, depressed or hopeless	**0**	**1**	**2**	**3**

Describe HOW YOU FEEL RIGHT NOW *by circling the most appropriate number below***:**

	Not at all	A little	Moderate	Quite a bit	Extremely
Confusion	**1**	**2**	**3**	**4**	**5**

Please detail what you feel confused about below (if applicable):

Time ☐ Tests/ assessments ☐ Place ☐

Other (please provide details below):

……
……

Over the last 4 hours has there been anything that has distracted you?

☐ Light

☐ People

☐ Sound

☐ Other (if selecting this box please detail the nature of the distraction below)

……
……

Study team to record

Exercise taken: ☐ *Yes* ☐ No *Details*: …… *Duration:* ……

Lab form 2

*Subject code:_____ Date:_____ Time: _____(*To be completed by study team)*

This form is to be completed after each sleep episode/ nap.
Please indicate whether you think the time is/was am or pm and provide an estimate of the time for the following questions.

			Study team to record time	
What time did you go to bed?		AM/PM		AM/PM
What time did you try starting to sleep at?		AM/PM		AM/PM
How long did it take you to fall asleep? (min)				
How many times did you wake up?				
How long were you awake for? (min) Please estimate the time and duration of each night awakening.				
What time did you wake up?		AM/PM		AM/PM
What time did you get up?		AM/PM		AM/PM

How would you rate your quality of sleep?

1 2 3 4 5 6 7 8 9

Best sleep ever. **Worst sleep ever**

How difficult did you find to wake up/get up?

1 2 3 4 5 6 7 8 9

Very easy **Quite hard**

1. Do you recall your dream/s from your last sleep episode/nap?

   Yes ☐ No ☐

If yes, how intense were your dreams emotionally *(please tick the box that applies)?*

Not at all intense	Not that intense	Somewhat intense	Quite intense	Very intense
☐	☐	☐	☐	☐

2. What **was** the emotional tone of your dream/s on average **(please tick the box that applies)?**

Very negative	Somewhat negative	Neutral	Somewhat positive	Very positive
☐	☐	☐	☐	☐

3. Did you experience a so-called lucid dream/s (see definition)? Definition: In a lucid dream, one is aware that one is dreaming during the dream. Thus it is possible to wake up deliberately, or to influence the action of the dream actively, or to observe the course of the dream passively.

☐ Yes ☐ No

If **yes** and you feel comfortable providing details please provide these below:

……
……
…….....
.....

4. Did you dream about the lab session?

☐ Yes ☐ No

If yes, please indicate what you dreamt about below:

☐ the tests ☐ the lab itself ☐ the people in the lab

If other please detail below if you are happy to disclose this:

……
……
…….....

Lab form 3

Subject code:_____ Date:_____ Time: _____(To be completed by study team)

KSS Instructions:

Please indicate your level of sleepiness for the previous 5 minutes using the scale below.

Extremely alert	1
	2
Alert	3
	4
Neither sleepy nor alert	5
	6
Sleepy, but not fighting sleep	7
	8
Extremely sleepy; it is an effort to stay awake	9

Answer the following questions by placing a vertical mark through the line for each question. Mark the line according to how you feel at this moment. Regard both the ends of the lines as indicating the most extreme sensations you have ever felt.

How hungry do you feel?

I am not hungry at all — I have never been more hungry

How thirsty do you feel?

Not at all thirsty — Extremely thirsty

Would you like to eat something sweet?

No, not at all — Yes, very much

Would you like to eat something salty?

No, not at all — Yes, very much

Would you like to eat something savoury?

No, not at all — Yes, very much

Would you like to eat something fatty?

No, not at all — Yes, very much

This scale below consists of words that describe different feelings and emotions. *Read each item and then circle the appropriate number next to the word indicating the extent to which you feel this way*

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Enthusiastic	**1**	**2**	**3**	**4**	**5**
Irritable	**1**	**2**	**3**	**4**	**5**

Please indicate how you feel at the moment by placing a vertical mark on the scales below for the following items. Regard both the ends of the lines as indicating the most extreme sensations you have ever felt.

Mood

Worst ever ——— Best ever

Tension

Worst ever ——— Best ever

Physical comfort

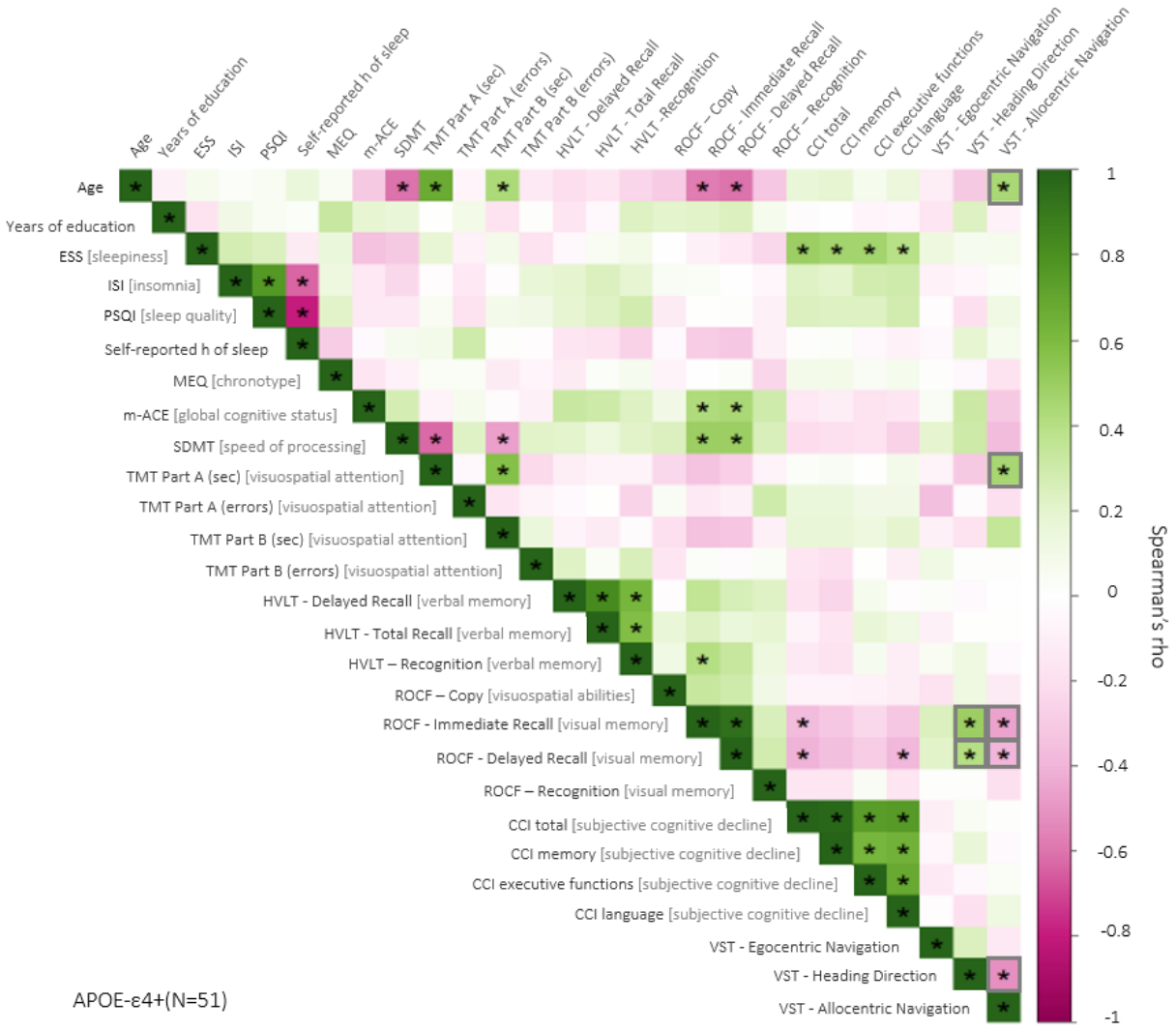
Worst ever ——— Best ever

Study team to record

Blood pressure= ……

Temperature=…….

Appendix 10 - Associations between self-reported sleep, chronotype and cognitive measures in APOE-ε4+ and non-carriers



A. APOE-ε4+(N=51)

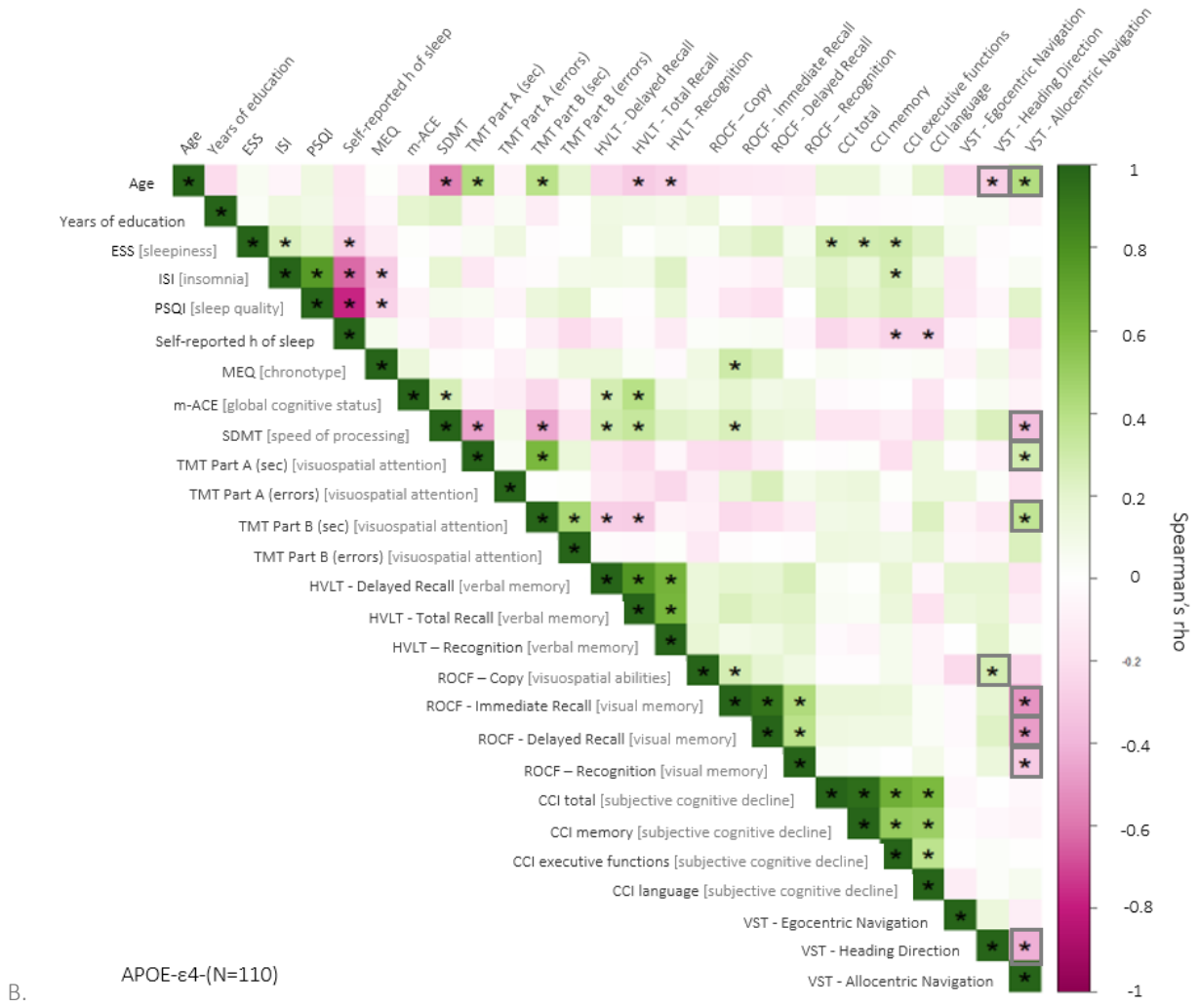
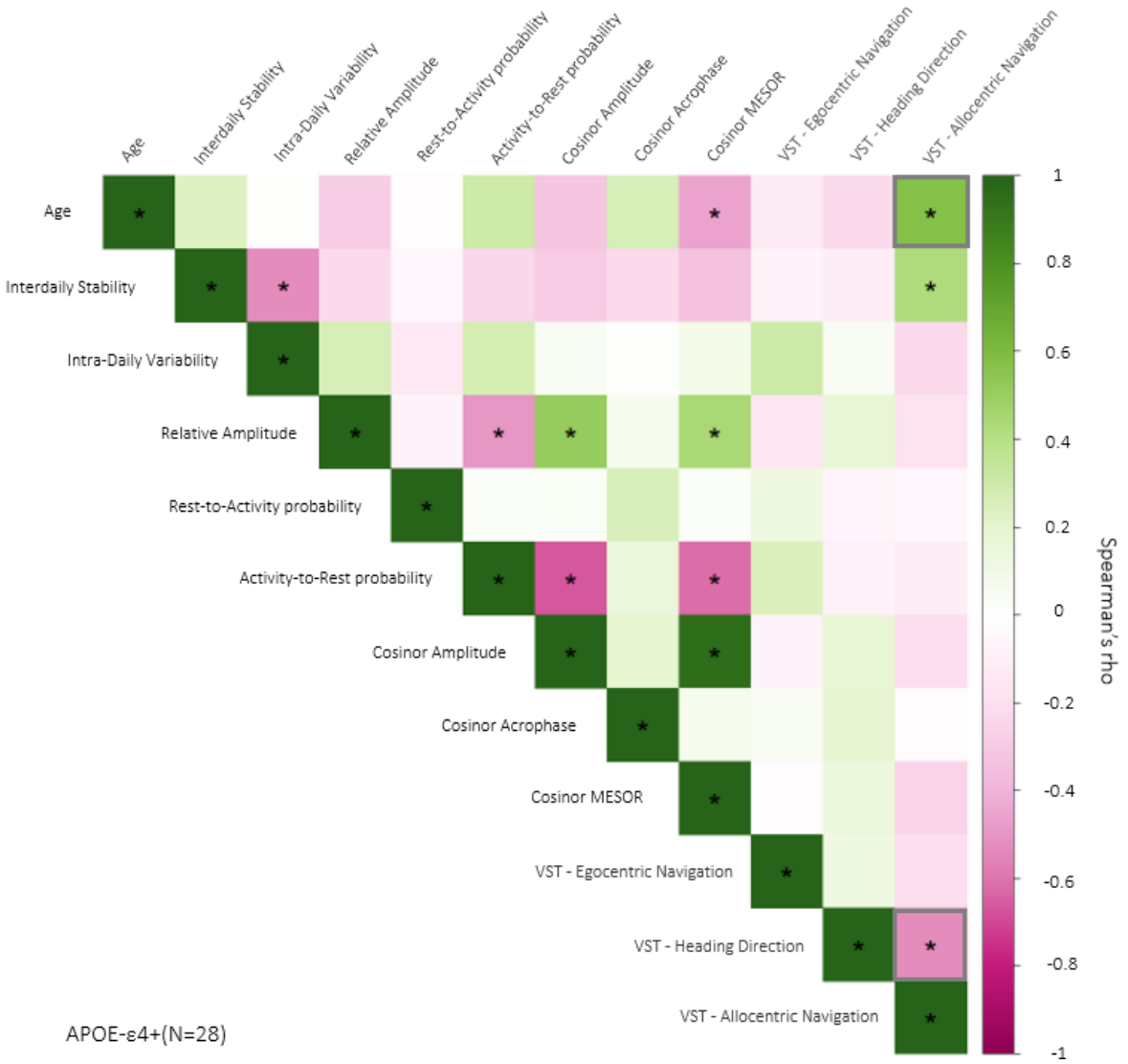
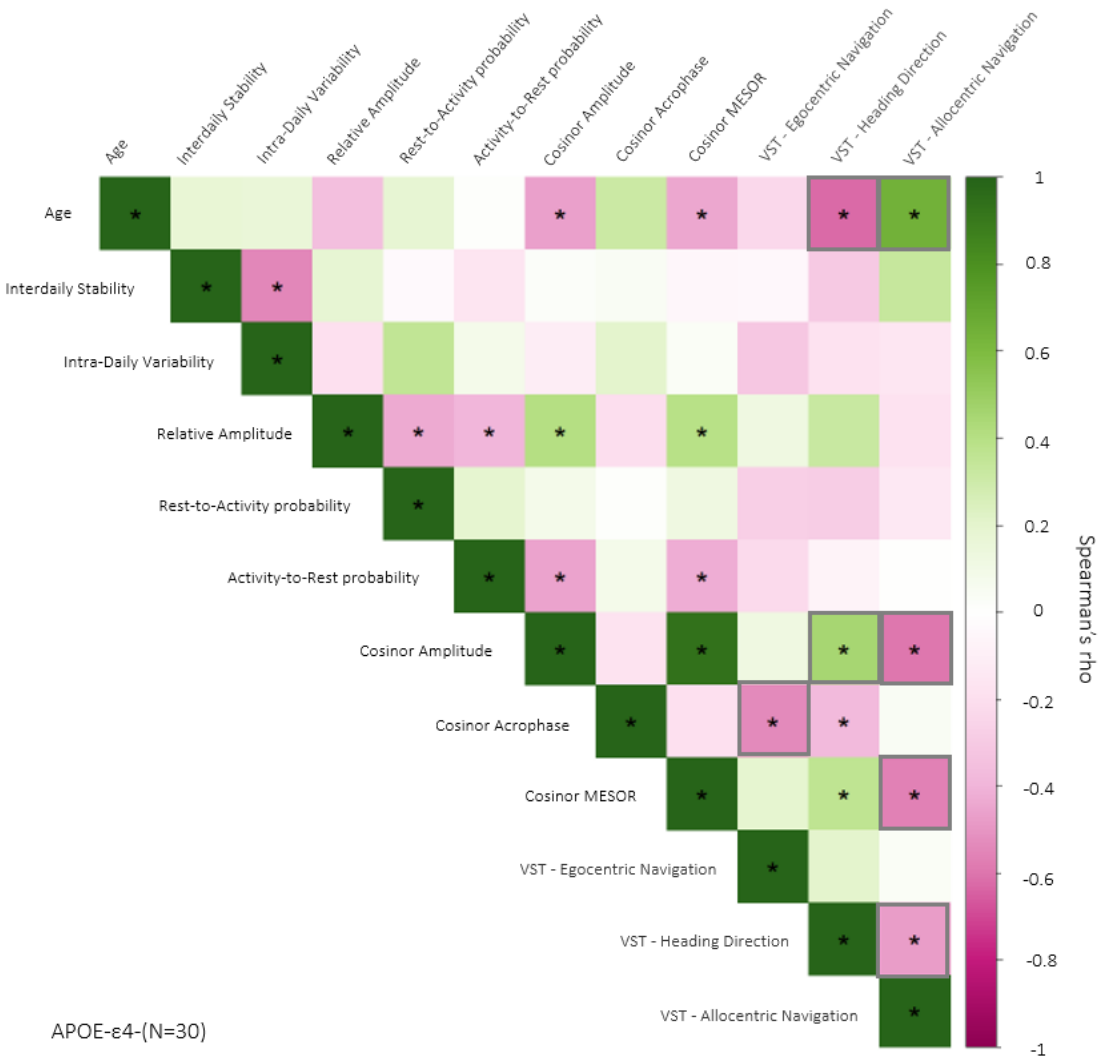


Figure 1. Adjacency matrixes showing associations between self-reported sleep, chronotype and cognitive measures in APOE-ε4+ (n=51) (A.) and non-carriers (n=110) (B). Stars indicate significant correlations (p<0.01). Colour scale indicates the strength of correlation. The further Spearman's ρ is from zero, the stronger is the association between the variables. The sign of ρ indicates the direction of the relationship, i.e., if it is positive (green), then as one variable increase, the other does likewise. Significant ($p \leq 0.05$) VST correlations that survived Benjamin-Hochberg correction are highlighted in grey.

Appendix 11 - Associations between actigraphy-based rest-activity variables and the Supermarket task in APOE-ε4+ and non-carriers



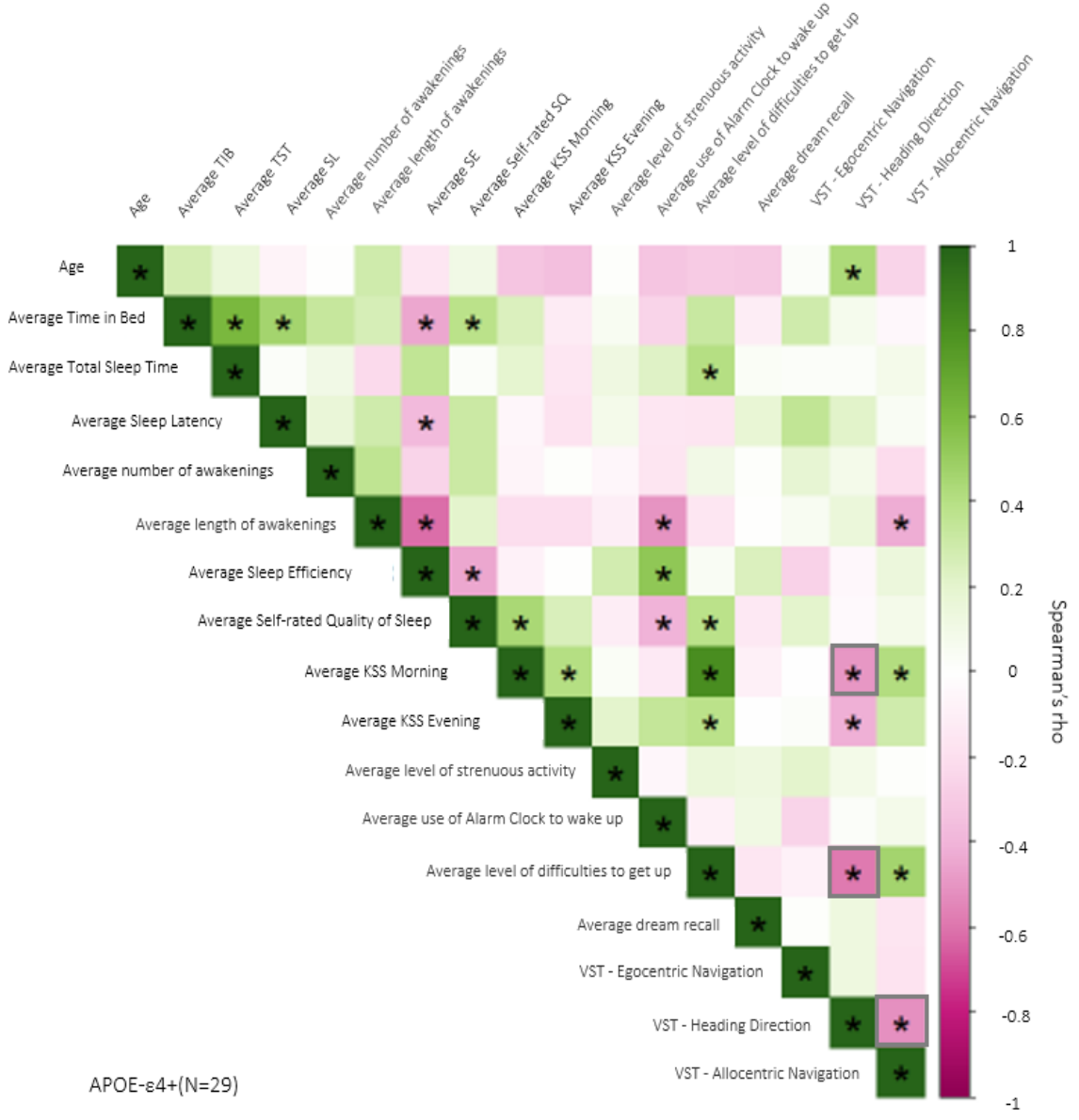
A. APOE-ε4+(N=28)



B.

Figure 1. Adjacency matrixes showing associations between rest-activity variables and the Supermarket task in APOE-ε4+ (n=28) (A) and non-carriers (n=30) (B). Stars indicate significant correlations (p<0.01). Colour scale indicates the strength of correlation. The further Spearman's ρ is from zero, the stronger is the association between the variables. The sign of ρ indicates the direction of the relationship, i.e., if it is positive (green), then as one variable increase, the other does likewise. Significant ($p\leq0.05$) correlations that survived Benjamin-Hochberg correction are highlighted in grey.

Appendix 12 - Associations between sleep diary-based outcome measures and the Supermarket task in APOE-ε4+ and non-carriers



A. APOE-ε4+(N=29)

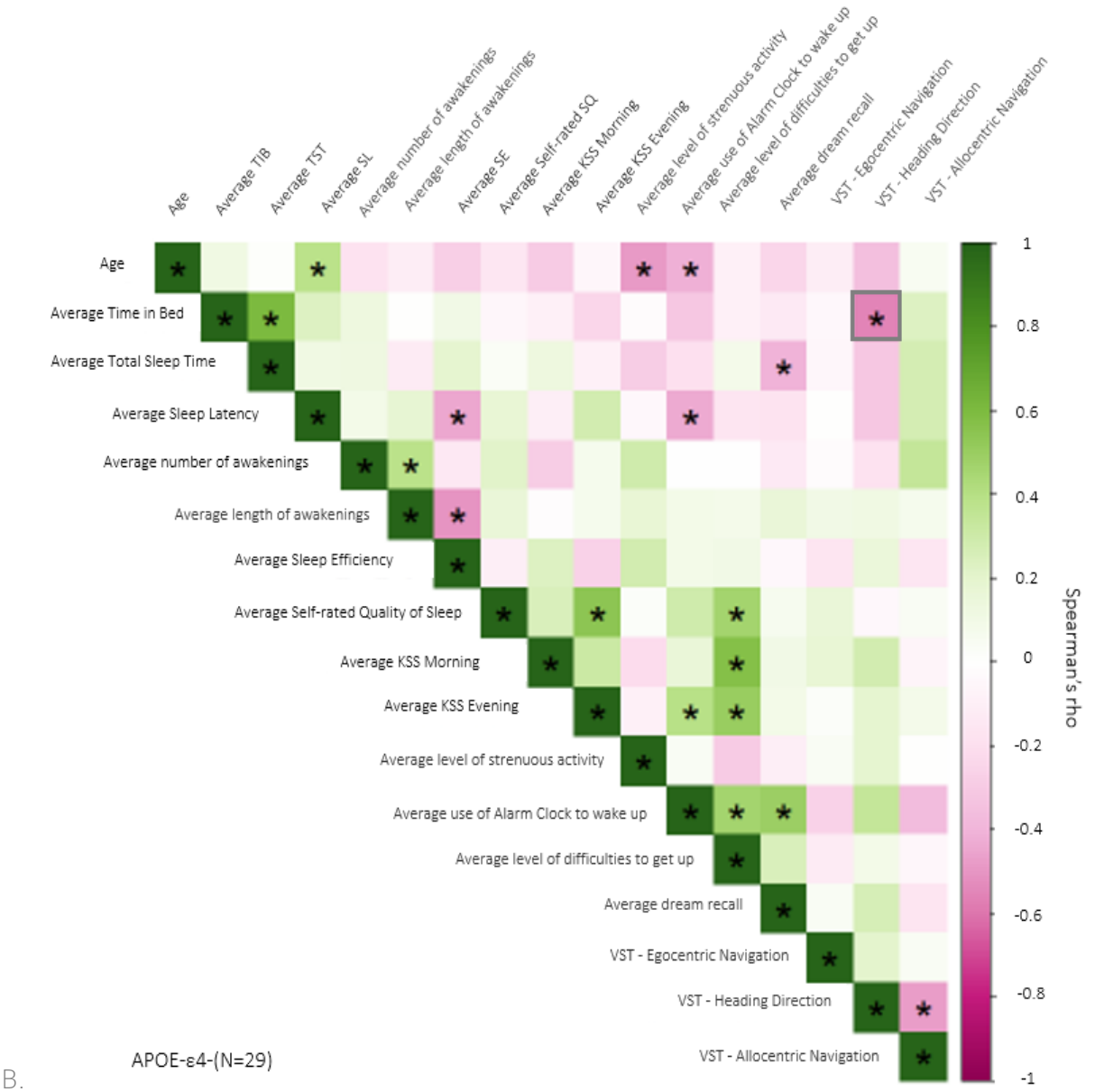
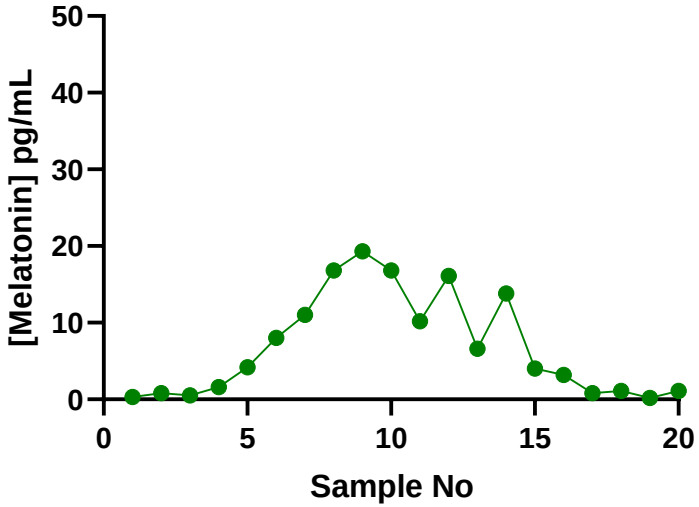
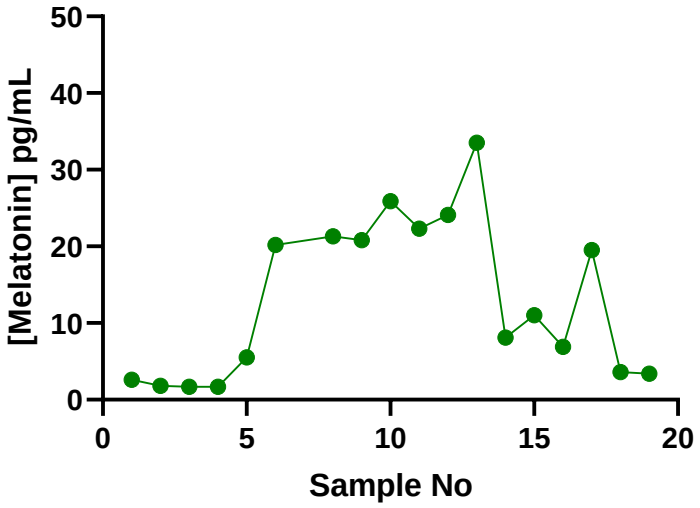


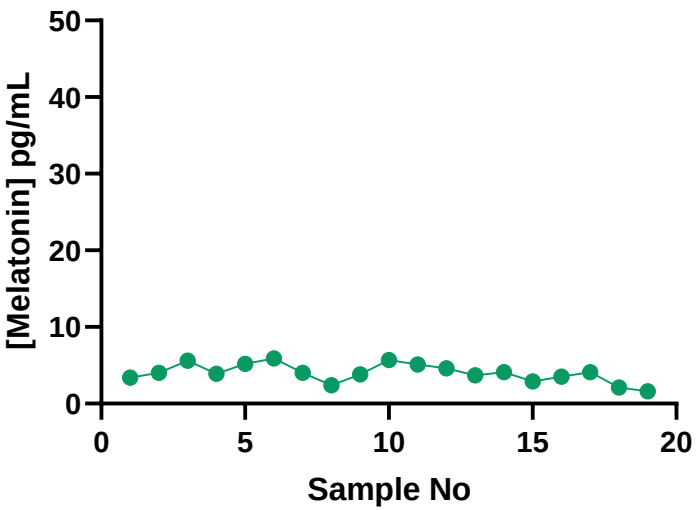
Figure 1. Adjacency matrixes showing associations between sleep dairy outcome measures and the Supermarket task in APOE-ε4+ (n=29) (A) and non-carriers (n=29) (B). Stars indicate significant correlations (p<0.01). Colour scale indicates the strength of correlation. The further Spearman's ρ is from zero, the stronger is the association between the variables. The sign of ρ indicates the direction of the relationship, i.e., if it is positive (green), then as one variable increase, the other does likewise. Significant ($p \leq 0.05$) correlations that survived Benjamin-Hochberg correction are highlighted in grey.



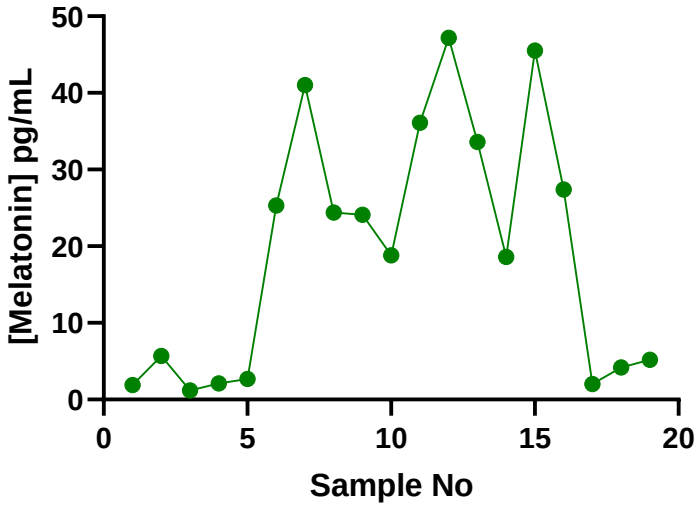
APO015 - M, 77, ε4⁻, SD



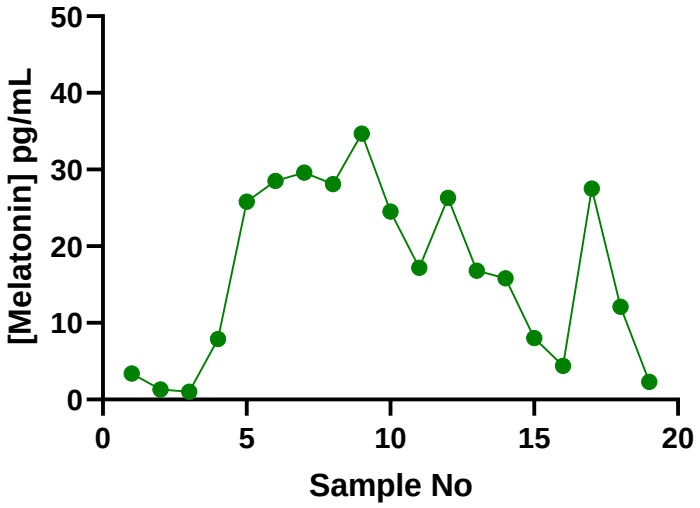
APO016 - F, 78, ε4⁺, SD



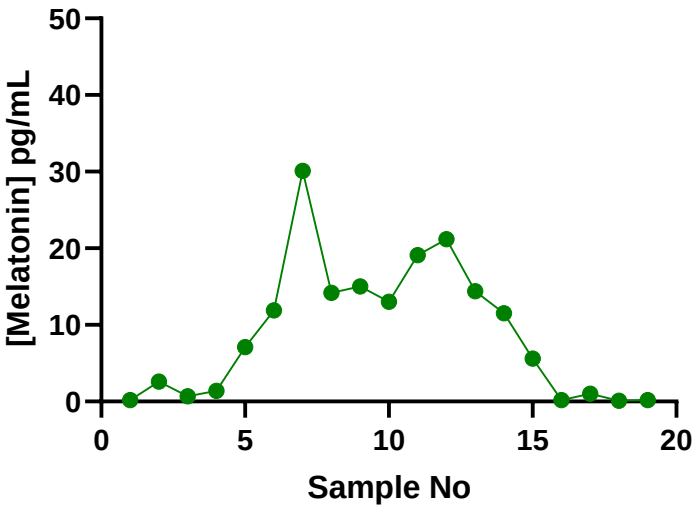
APO025 - 58, M, ε4⁻, SD



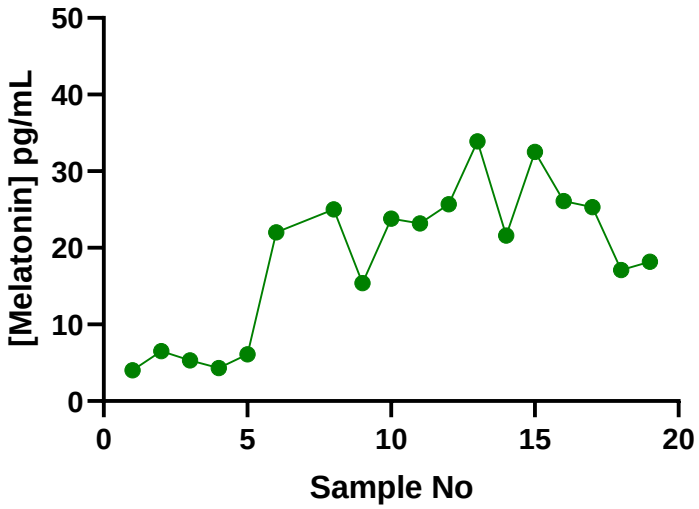
APO046 - F, 66, ε4⁻, SD



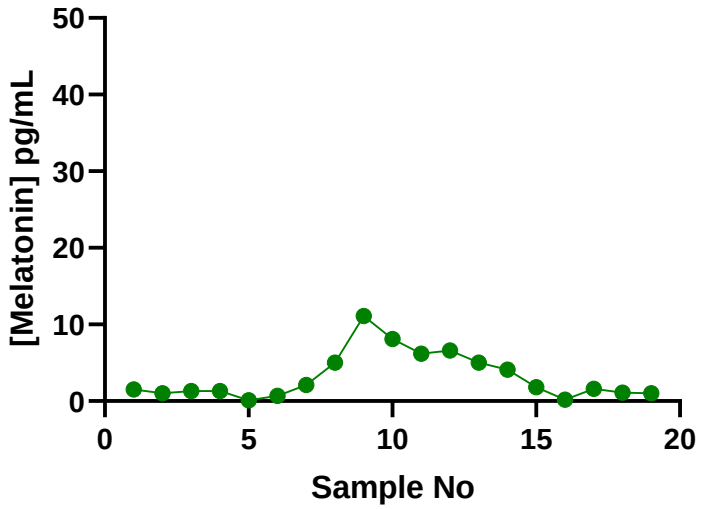
APO076 - F, 69, ε4⁻, SD



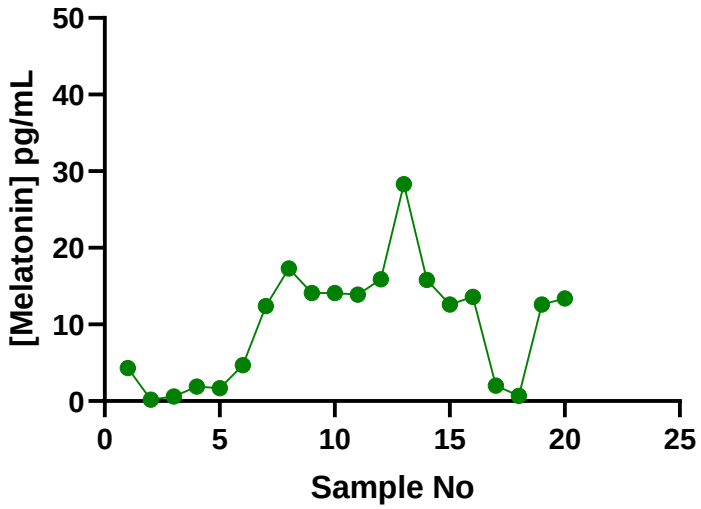
APO087 - M, 59, ε4-, SD



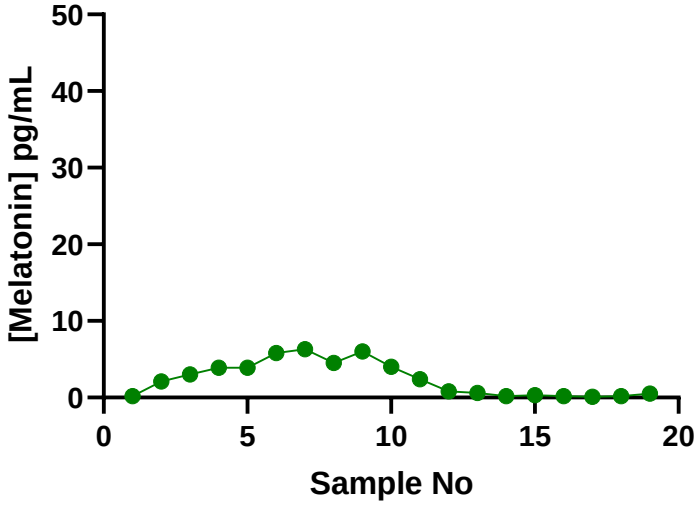
APO088 - M, 68, ε4+, SD



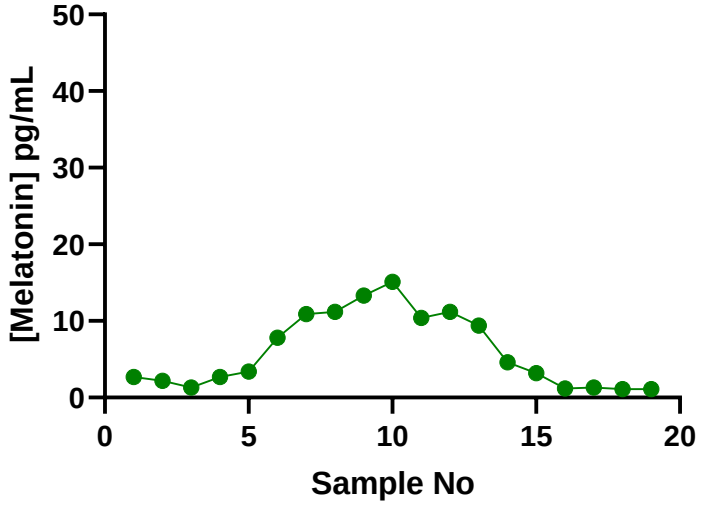
APO089 - F, 51, ε4-, SD



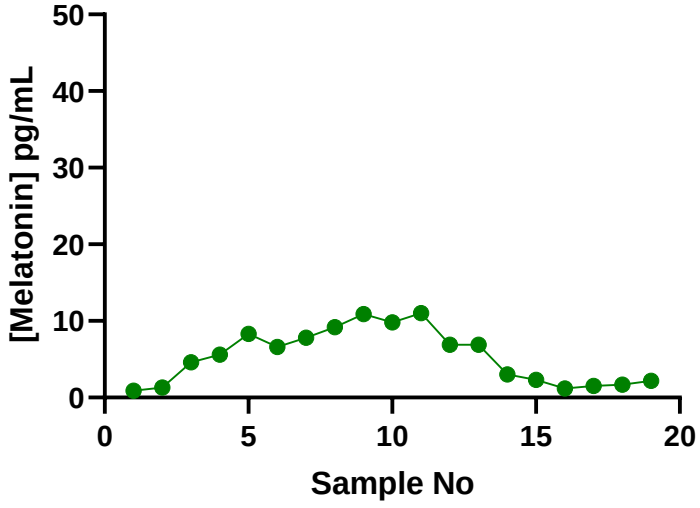
APO098 - F, 66, ε4-, SD



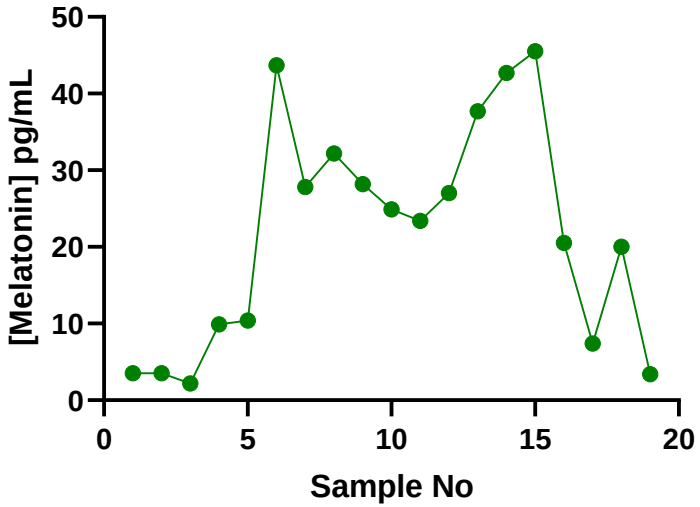
APO108 - M, 47, ε4-, SD



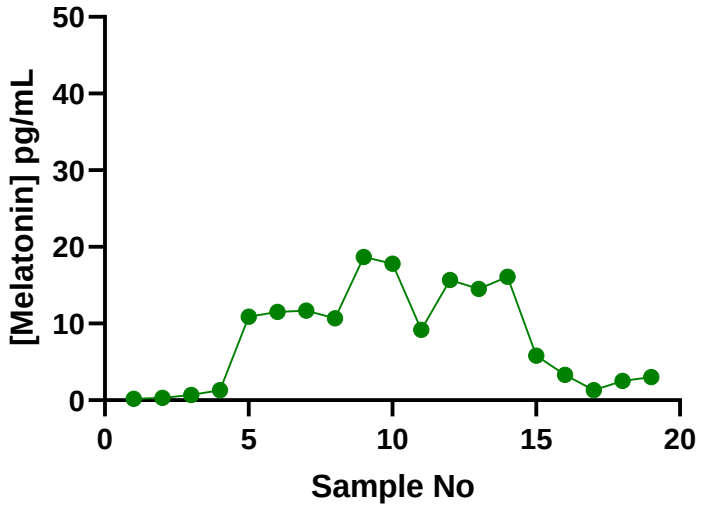
APO109 - F, 60, ε4-, SD



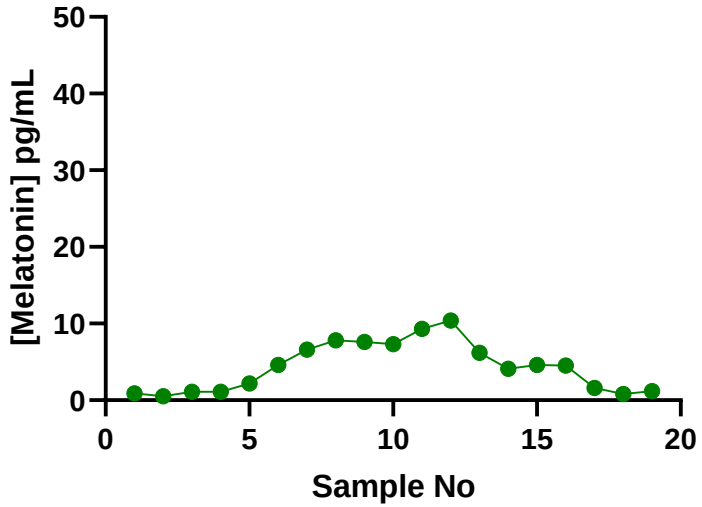
APO121 - F, 59, ε4+, SD



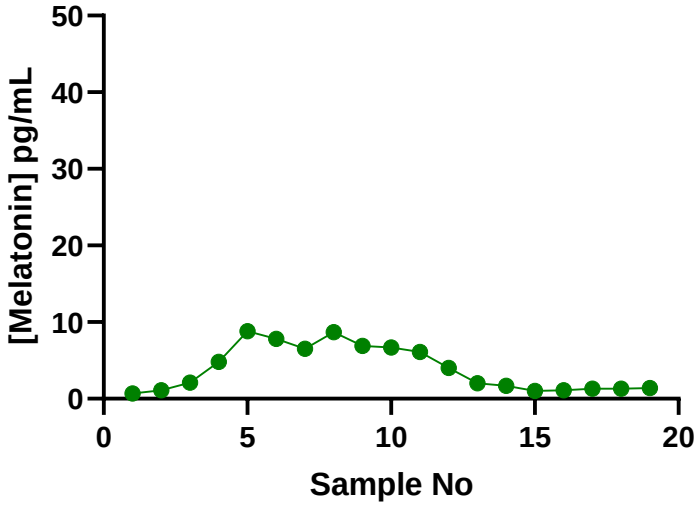
APO127 - F, 74, ε4+, SD



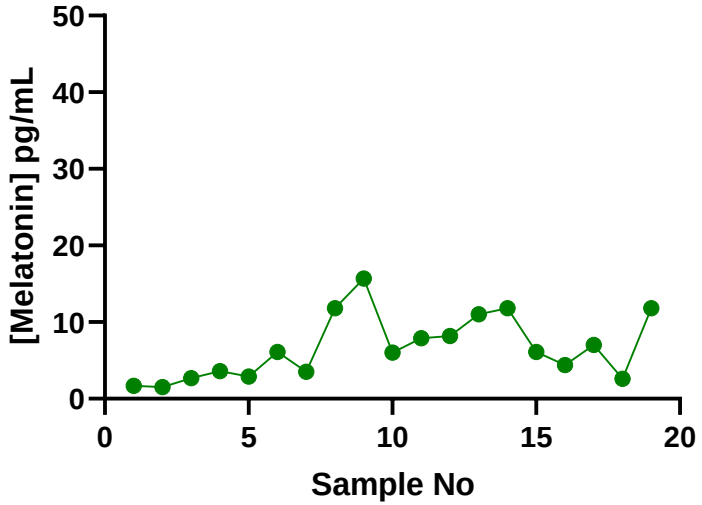
APO134 - M, 61, ε4+, SD



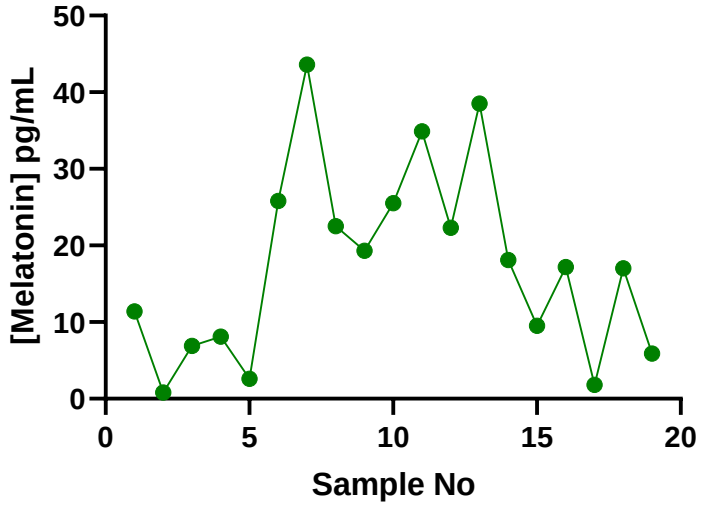
APO136 - F, 60, ε4+, SD



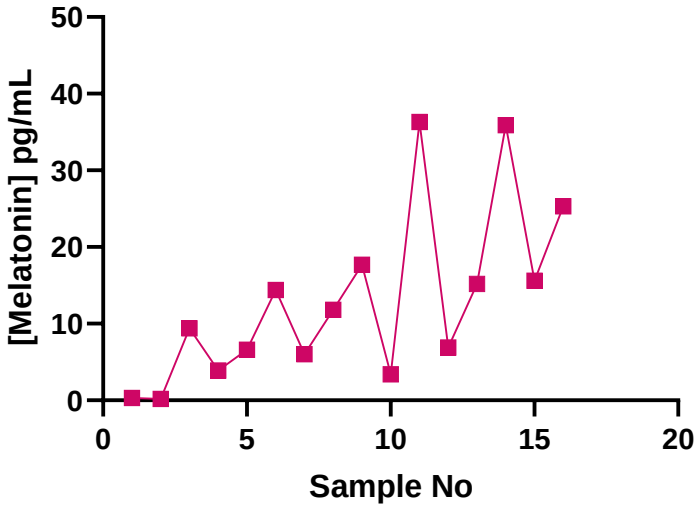
APO142 - M, 73, ε4+, SD



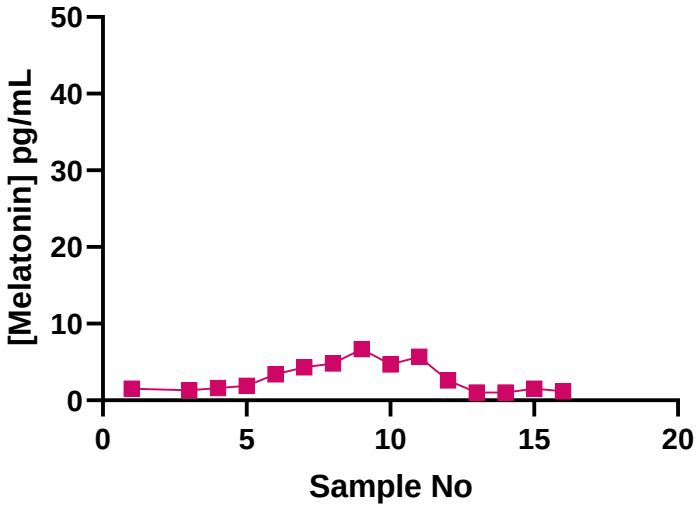
APO167- M, 54, ε4+, SD



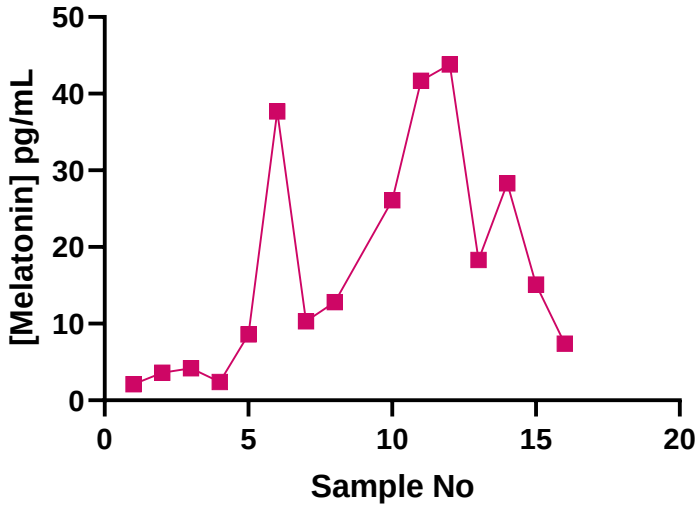
APO003 - M, 70, ε4^{-}, MN



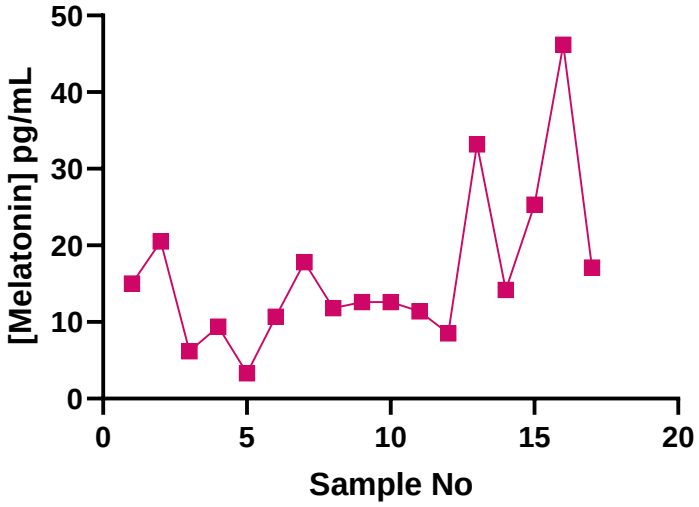
APO005 - M, 67, ε4^{+}, MN



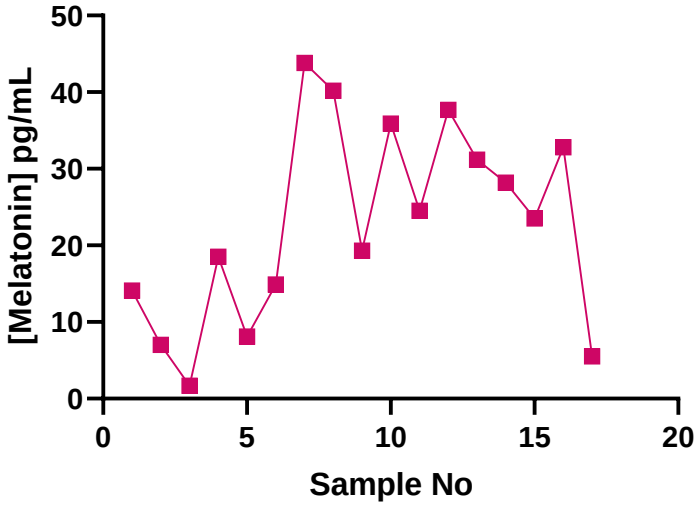
APO008 - F, 62, ε4^{+}, MN



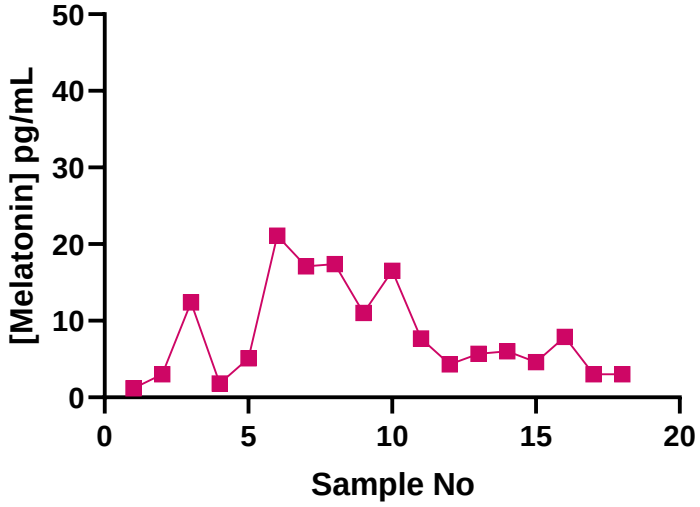
APO010 - F, 44, ε4^{+}, MN



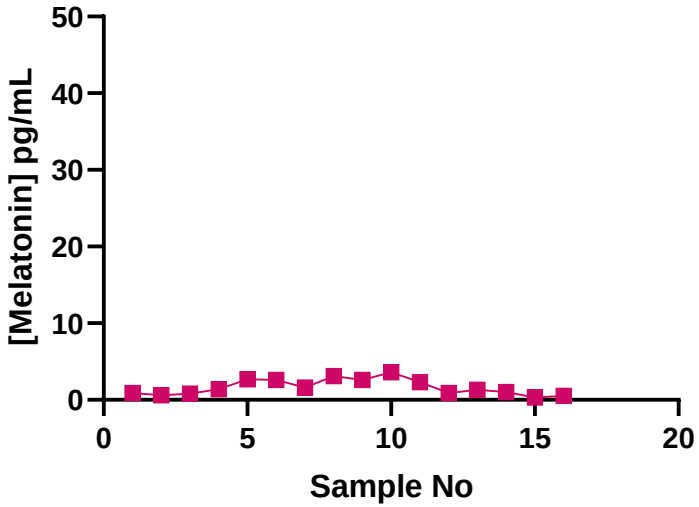
APO024 - M, 61, ε4^{+}, MN



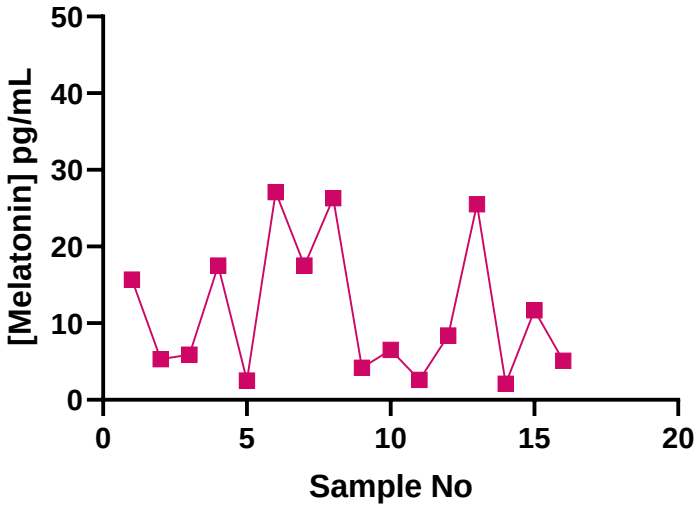
APO027 - 63, M, ε4^{-}, MN



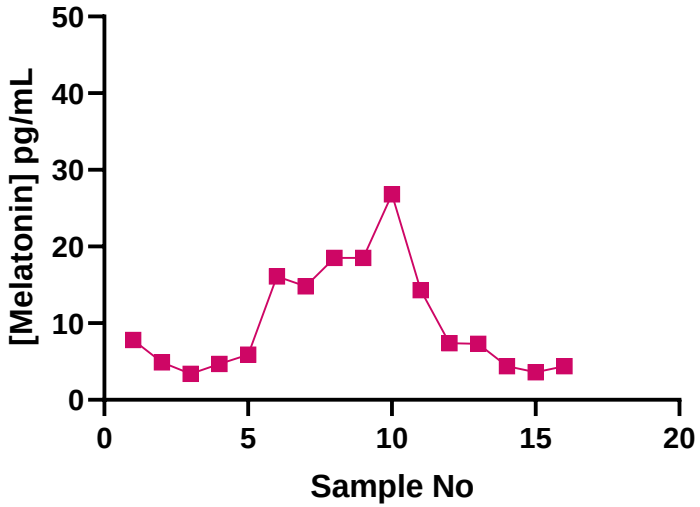
APO042 - F, 74, ε4-, MN



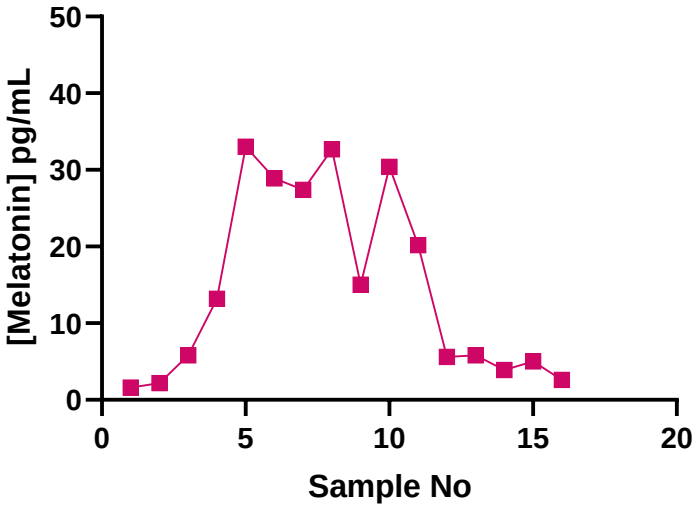
APO053 - F, 59, ε4-, MN



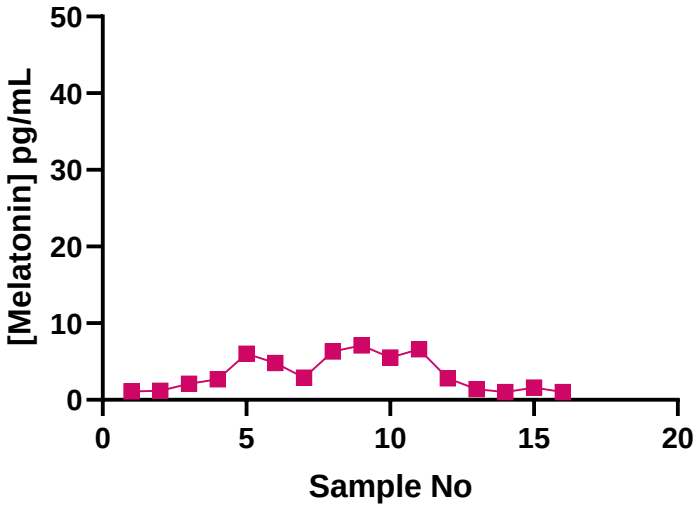
APO068 - M, 61, ε4-, MN



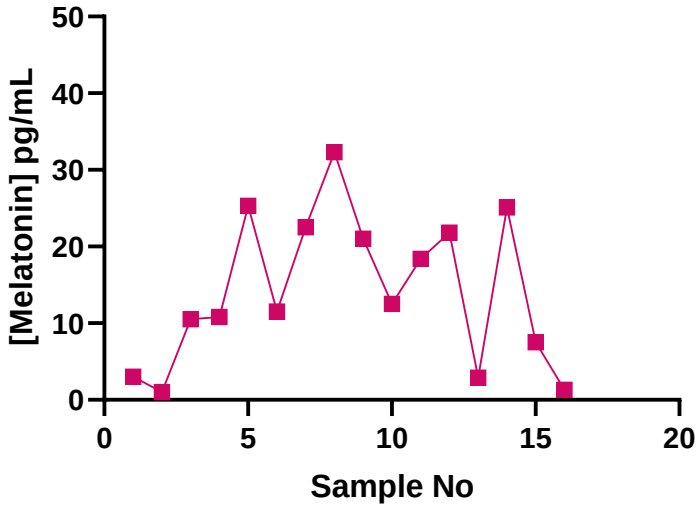
APO097 - F, 71, ε4-, MN



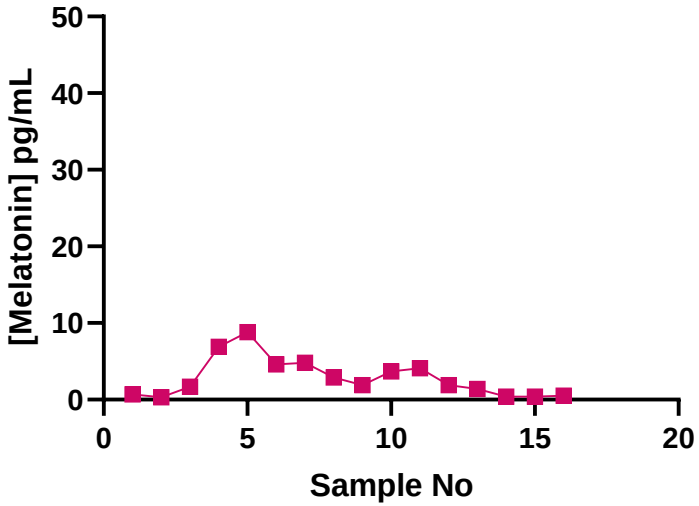
APO102 - F, 74, ε4-, MN



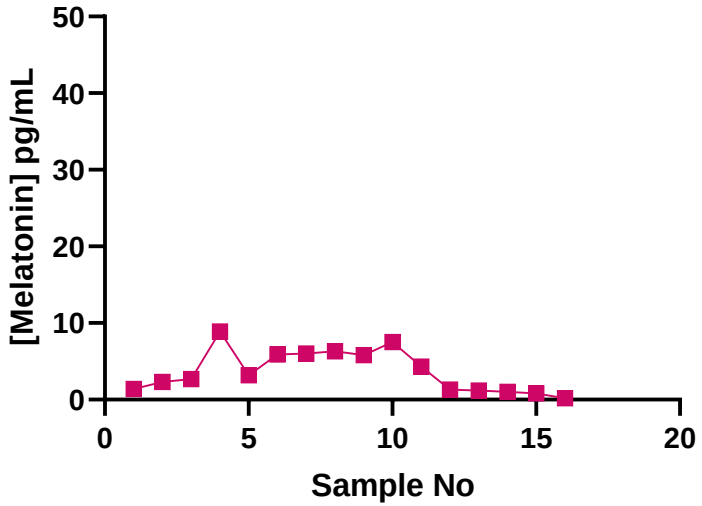
APO104 - F, 59, ε4-, MN



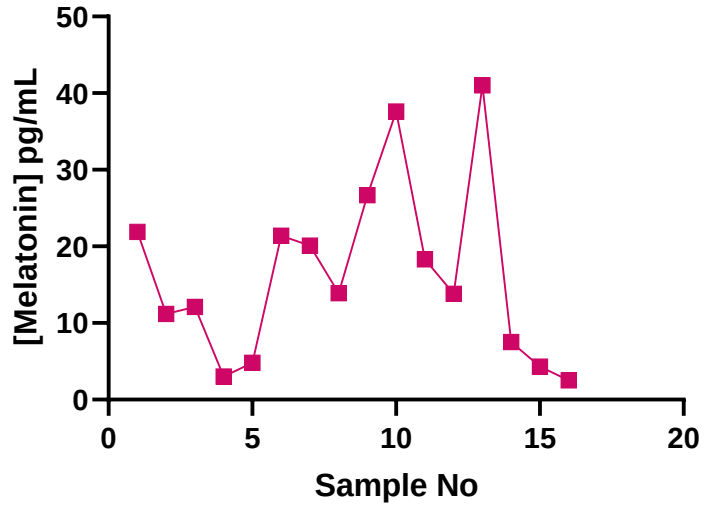
APO129 - F, 73, ε4+, MN



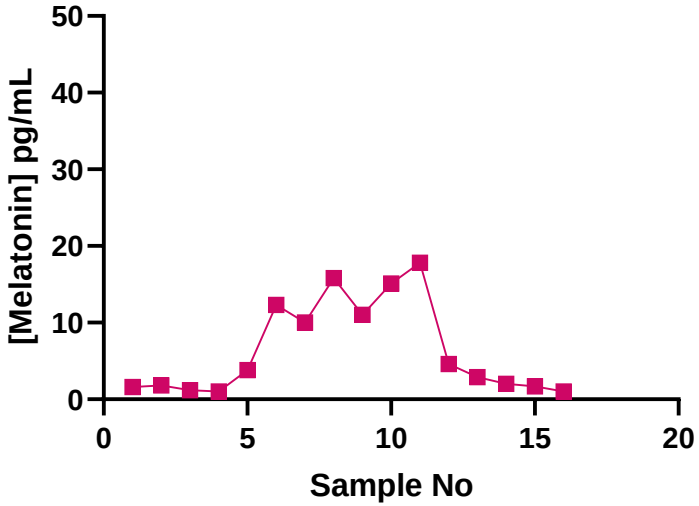
APO130 - M, 63, ε4+, MN



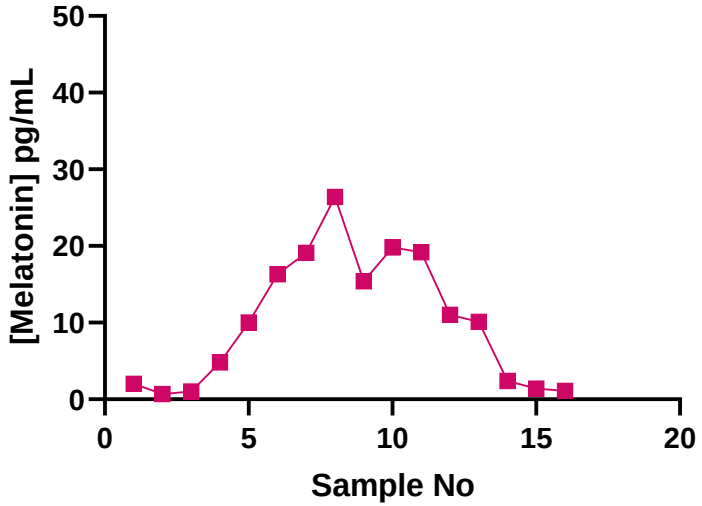
APO158 - M, 67, ε4+, MN



APO162 - F, 52, ε4+, MN



APO169 - M, 63, ε4+, MN



Appendix 14 - *Statistics for Model 1*

Statistics for each model – KSS and PVT (Model 1)

KSS

variable	*F*	*p*	η^2
session number	12.31	<0.001	0.28
sex	6.93	0.01	0.19
age	2.99	0.09	0.09
hours of sleep at Baseline Night	2.01	0.17	0.07
protocol*session number	16.16	<0.001	0.06
protocol	0.40	0.53	0.004
session number	12.31	<0.001	0.28

PVT – Mean RT (no lapses)

variable	*F*	*p*	η^2
session number	10.62	<0.0001	0.25
years of education	5.18	0.03	0.16
protocol*session number	26.17	<0.0001	0.09
sex	0.18	0.67	0.01
age	0.10	0.75	0.00
hours of sleep at Baseline Night	0.01	0.91	0.00
protocol	0.02	0.88	0.00

PVT – number of lapses

variable	*F*	*p*	η^2
age	7.58	0.01	0.21
session number	4.36	<0.0001	0.12
Protocol*session number	13.97	<0.0001	0.05
protocol	0.78	0.38	0.01
sex	0.18	0.67	0.01
years of education	0.05	0.83	0.00
hours of sleep at Baseline Night	0.00	1.00	0.00

Statistics for each model – n-back (Model 1)

One-back – Reaction Time (RT)

variable	*F*	*p*	η^2
session number	6.96	<0.0001	0.22
years of education	0.57	0.46	0.02
protocol*session number	4.06	0.05	0.02
hours of sleep at Baseline Night	0.46	0.50	0.02
age	0.10	0.76	0.00
protocol	0.01	0.94	0.00
sex	0.00	0.98	0.00

Two-back RT – Reaction Time (RT)

variable	*F*	*p*	η^2
session number	0.93	0.49	0.04
sex	0.73	0.40	0.03
age	0.56	0.46	0.02
protocol	0.18	0.68	0.00
hours of sleep at Baseline Night	0.06	0.81	0.00
years of education	0.00	0.98	0.00
protocol*session number	0.01	0.94	0.00

One-back – Accuracy (ACC)

variable	*F*	*p*	η^2
session number	2.09	0.04	0.08
hours of sleep at Baseline Night	0.78	0.39	0.03
age	0.54	0.47	0.02
years of education	0.38	0.54	0.02
protocol*session number	1.21	0.27	0.01
sex	0.13	0.72	0.01
protocol	0.03	0.87	0.00

Two-back – Accuracy (ACC)

variable	*F*	*p*	η^2
session number	9.59	<0.0001	0.28
protocol*session number	25.88	<0.0001	0.11
protocol	4.80	0.03	0.10
years of education	0.88	0.36	0.04
hours of sleep at Baseline Night	0.62	0.44	0.03
sex	0.30	0.59	0.01
age	0.10	0.75	0.00

Statistics for each model – subjective cognitive demand and mental effort (Model 1)

Cognitive demand – the whole session

variable	*F*	*p*	η^2
session number	3.11	<0.0001	0.09
age	2.31	0.14	0.08
years of education	1.52	0.23	0.06
protocol*session number	11.65	0.002	0.05
sex	1.07	0.31	0.04
hours of sleep at Baseline Night	0.11	0.75	0.00
protocol	0.01	0.93	0.00

Mental effort - PVT

variable	*F*	*p*	η^2
age	3.48	0.07	0.11
protocol	5.35	0.03	0.10
sex	2.06	0.16	0.07
session number	2.43	0.02	0.07
protocol*session number	4.69	0.03	0.02
hours of sleep at Baseline Night	0.18	0.68	0.01
years of education	0.05	0.83	0.00

Statistics for each model – Episodic Memory task (Model 1)

Episodic memory – time to complete

variable	*F*	*p*	η^2
age	0.50	0.49	0.02
session number	0.26	0.98	0.01
protocol	0.50	0.48	0.01
protocol*session number	1.72	0.19	0.01
years of education	0.26	0.61	0.01
sex	0.29	0.60	0.01
hours of sleep at Baseline Night	0.30	0.59	0.01

Episodic memory – Recognition – Hits – time to complete

variable	*F*	*p*	η^2
years of education	6.95	0.01	0.22
age	1.10	0.30	0.04
session number	0.83	0.57	0.03
sex	0.68	0.42	0.03
hours of sleep at Baseline Night	0.60	0.45	0.02
protocol	1.20	0.28	0.02
protocol*session number	0.02	0.89	0.00

Episodic memory – Recognition – Correct Rejections – time to complete

variable	*F*	*p*	η^2
protocol	3.30	0.08	0.08
age	1.59	0.22	0.06
session number	1.17	0.32	0.04
hours of sleep at Baseline Night	0.94	0.34	0.04
protocol*session number	8.67	0.004	0.04
years of education	0.19	0.66	0.01
sex	0.05	0.82	0.00

Episodic memory – Source Memory – Hits

variable	*F*	*p*	η^2
years of education	5.18	0.03	0.17
sex	2.66	0.12	0.10
session number	0.50	0.86	0.02
protocol	1.18	0.28	0.01
hours of sleep at Baseline Night	0.26	0.61	0.01
protocol*session number	2.24	0.14	0.01
age	0.05	0.82	0.00

Episodic memory – Source Memory – time to complete

variable	*F*	*p*	η^2
years of education	1.03	0.32	0.04
sex	0.94	0.34	0.04
session number	0.67	0.72	0.02
protocol*session number	0.81	0.37	0.00
hours of sleep at Baseline Night	0.06	0.80	0.00
age	0.05	0.82	0.00
protocol	0.00	0.99	0.00

Statistics for each model – SHQ – allocentric navigation (Model 1)

SHQ – Wayfinding – distance to travel – Easy levels

variable	*F*	*p*	η^2
age	0.72	0.41	0.03
session number	0.75	0.65	0.03
sex	0.44	0.52	0.02
protocol*session number	2.59	0.11	0.01
years of education	0.18	0.67	0.01
protocol	0.10	0.76	0.00
hours of sleep at Baseline Night	0.00	0.98	0.00

SHQ – Wayfinding – distance to travel – Hard levels

variable	*F*	*p*	η^2
session number	0.75	0.64	0.03
hours of sleep at Baseline Night	0.55	0.47	0.03
sex	0.42	0.52	0.02
age	0.03	0.86	0.00
protocol	0.03	0.87	0.00
years of education	0.01	0.94	0.00
protocol*session number	0.02	0.90	0.00

SHQ – Wayfinding – time to complete – Easy levels

variable	*F*	*p*	η^2
age	1.13	0.30	0.05
years of education	0.58	0.46	0.03
sex	0.53	0.47	0.02
session number	0.64	0.74	0.02
protocol*session number	4.24	0.04	0.02
hours of sleep at Baseline Night	0.14	0.71	0.01
protocol	0.10	0.76	0.00

SHQ – Wayfinding – time to complete – Hard levels

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	1.93	0.18	0.08
sex	0.62	0.44	0.03
session number	0.71	0.69	0.03
protocol	0.04	0.84	0.00
age	0.01	0.94	0.00
protocol*session number	0.01	0.91	0.00
years of education	0.00	1.00	0.00

SHQ – Wayfinding – Mental effort – Easy levels

variable	*F*	*p*	η^2
age	4.81	0.04	0.16
sex	4.68	0.04	0.16
protocol	3.61	0.06	0.08
hours of sleep at Baseline Night	0.59	0.45	0.02
years of education	0.58	0.45	0.02
session number	0.37	0.94	0.01
protocol*session number	0.59	0.44	0.00

SHQ – Wayfinding – Mental effort – Hard levels

variable	*F*	*p*	η^2
age	4.24	0.05	0.15
sex	3.32	0.08	0.12
protocol	1.97	0.17	0.05
hours of sleep at Baseline Night	1.13	0.30	0.04
session number	0.71	0.68	0.02
years of education	0.09	0.76	0.00
protocol*session number	0.23	0.63	0.00

Statistics for each model – SHQ – egocentric navigation (Model 1)

SHQ – Flare – Accuracy – Easy levels

variable	*F*	*p*	η^2
protocol	0.54	0.47	0.05
hours of sleep at Baseline Night	0.00	1.00	0.02
age	2.86	0.10	0.02
protocol*session number	1.25	0.26	0.02
session number	1.49	0.16	0.02
years of education	0.00	0.98	0.00
sex	0.24	0.63	0.00

SHQ – Flare – Accuracy – Hard levels

variable	*F*	*p*	η^2
age	1.77	0.20	0.07
session number	0.89	0.53	0.03
protocol*session number	5.99	0.02	0.03
years of education	0.49	0.49	0.02
hours of sleep at Baseline Night	0.11	0.74	0.01
sex	0.08	0.78	0.00
protocol	0.08	0.78	0.00

SHQ – Flare – time to complete – Easy levels

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	1.01	0.33	0.04
protocol*session number	3.18	0.08	0.01
session number	0.24	0.98	0.01
age	0.17	0.69	0.01
sex	0.17	0.69	0.01
protocol	0.21	0.65	0.01
years of education	0.02	0.88	0.00

SHQ – Flare – time to complete – Hard levels

variable	*F*	*p*	η^2
protocol	12.55	≤0.001	0.22
age	2.93	0.10	0.12
session number	3.45	0.001	0.11
protocol*session number	14.16	≤0.001	0.06
sex	0.78	0.39	0.03
years of education	0.10	0.76	0.00
hours of sleep at Baseline Night	0.00	0.96	0.00

SHQ – Flare – Mental effort – Easy levels

variable	*F*	*p*	η^2
age	3.98	0.06	0.14
protocol	4.25	0.05	0.10
sex	2.48	0.13	0.09
session number	1.37	0.21	0.04
hours of sleep at Baseline Night	0.21	0.65	0.01
years of education	0.04	0.84	0.00
protocol*session number	0.18	0.67	0.00

SHQ – Flare – Mental effort – Hard levels

variable	*F*	*p*	η^2
age	4.24	0.05	0.15
sex	3.32	0.08	0.12
protocol	1.97	0.17	0.05
hours of sleep at Baseline Night	1.13	0.30	0.04
session number	0.71	0.68	0.02
years of education	0.09	0.76	0.00
protocol*session number	0.23	0.63	0.00

Appendix 15 - *Statistics for Model 2*

Statistics for each model – KSS and PVT (Model 2)

KSS

variable	*F*	*p*	η^2
session number	9.14	<0.001	0.22
sex	6.40	0.02	0.19
age	3.09	0.09	0.10
hours of sleep at Baseline Night	2.05	0.16	0.07
APOE-ε4 status	2.64	0.11	0.03
protocol	2.50	0.12	0.03
APOE-ε4 status * protocol	2.06	0.16	0.02
APOE-ε4 status *session number	3.23	0.07	0.01
APOE-ε4 status * session number * protocol	2.31	0.13	0.01
protocol * session number	0.04	0.84	0.00

PVT – Mean RT

variable	*F*	*p*	η^2
years of education	5.98	0.02	0.19
session number	3.75	0.001	0.10
protocol * session number	5.23	0.02	0.02
age	0.41	0.53	0.02
protocol	0.48	0.49	0.01
APOE-ε4 status * protocol	0.46	0.50	0.01
APOE-ε4 status	0.30	0.59	0.01
APOE-ε4 status * protocol * session number	0.54	0.46	0.00
sex	0.04	0.84	0.00
hours of sleep at Baseline Night	0.02	0.90	0.00
APOE-ε4 status * session number	0.04	0.83	0.00

PVT – lapses

variable	*F*	*p*	η^2
age	6.86	0.01	0.21
session number	2.66	0.01	0.08
protocol * session number	10.54	0.001	0.04
protocol	3.93	0.05	0.04
APOE-ε4 status * protocol	3.32	0.07	0.03
APOE-ε4 status	1.95	0.17	0.02
APOE-ε4 status * protocol * session number	4.88	0.03	0.02
APOE-ε4 status * session number	1.65	0.20	0.01
sex	0.08	0.78	0.00
years of education	0.03	0.86	0.00
hours of sleep at Baseline Night	0.02	0.89	0.00

Statistics for each model – n-back (Model 2)

n-back – One-back RT

variable	*F*	*p*	η^2
session number	6.29	≤0.001	0.20
years of education	0.74	0.40	0.03
hours of sleep at Baseline Night	0.56	0.46	0.03
age	0.28	0.60	0.01
protocol * session number	2.46	0.12	0.01
APOE-ε4 status * protocol * session number	1.09	0.30	0.01
APOE-ε4 status	0.16	0.69	0.00
APOE-ε4 status * session number	0.44	0.51	0.00
sex	0.02	0.89	0.00
APOE-ε4 status * protocol	0.03	0.86	0.00
protocol	0.03	0.87	0.00

n-back – Two-back RT

variable	*F*	*p*	η^2
APOE-ε4 status	1.72	0.20	0.05
session number	1.12	0.35	0.04
protocol	1.30	0.26	0.04
APOE-ε4 status * protocol	1.07	0.31	0.03
sex	0.52	0.48	0.02
age	0.36	0.56	0.02
APOE-ε4 status * session number	1.45	0.23	0.01
protocol * session number	0.88	0.35	0.00
APOE-ε4 status * protocol * session number	0.87	0.35	0.00
years of education	0.03	0.87	0.00
hours of sleep at Baseline Night	0.00	0.99	0.00

n-back – One-back - Accuracy

variable	*F*	*p*	η^2
session number	1.39	0.20	0.05
age	1.13	0.30	0.05
APOE-ε4 status * protocol	3.23	0.08	0.03
protocol	2.71	0.10	0.02
APOE-ε4 status	2.49	0.12	0.02
sex	0.38	0.54	0.02
years of education	0.13	0.72	0.01
hours of sleep at Baseline Night	0.13	0.72	0.01
APOE-ε4 status * protocol * session number	0.58	0.45	0.00
APOE-ε4 status * session number	0.31	0.58	0.00
protocol * session number	0.11	0.74	0.00

n-back – Two-back - Accuracy

variable	*F*	*p*	η^2
session number	7.55	≤0.001	0.23
hours of sleep at Baseline Night	0.85	0.37	0.04
years of education	0.67	0.42	0.03
sex	0.29	0.60	0.01
protocol * session number	2.72	0.10	0.01
APOE-ε4 status	0.56	0.46	0.01
APOE-ε4 status * protocol	0.51	0.48	0.01
APOE-ε4 status * protocol * session number	0.02	0.88	0.00
age	0.00	0.97	0.00
protocol	0.00	0.95	0.00
APOE-ε4 status * session number	0.01	0.93	0.00

Statistics for each model – Mental effort (Model 2)

Cognitive demand – the whole session

variable	*F*	*p*	η^2
age	1.69	0.21	0.07
APOE-ε4 status * session number	14.13	≤0.001	0.05
years of education	1.28	0.27	0.05
session number	1.56	0.14	0.05
APOE-ε4 status * protocol * session number	10.95	0.001	0.04
sex	0.77	0.39	0.03
protocol	0.64	0.43	0.02
APOE-ε4 status * protocol	0.60	0.45	0.02
APOE-ε4 status	0.56	0.46	0.02
protocol * session number	4.02	0.05	0.02
hours of sleep at Baseline Night	0.02	0.88	0.00

Mental effort - PVT

variable	*F*	*p*	η^2
APOE-ε4 status	3.78	0.06	0.10
APOE-ε4 status * protocol	2.42	0.13	0.07
protocol	2.34	0.14	0.07
age	1.45	0.24	0.05
session number	1.55	0.14	0.05
hours of sleep at Baseline Night	0.61	0.44	0.02
sex	0.61	0.44	0.02
APOE-ε4 status * session number	4.43	0.04	0.02
APOE-ε4 status * protocol * session number	1.88	0.17	0.01
years of education	0.08	0.78	0.00
protocol * session number	0.01	0.90	0.00

Statistics for each model – Episodic Memory task (Model 2)

Episodic memory – time to complete the whole task

variable	*F*	*p*	η^2
protocol	1.87	0.18	0.03
APOE-ε4 status * protocol	1.55	0.22	0.03
APOE-ε4 status	0.90	0.35	0.02
hours of sleep at Baseline Night	0.20	0.66	0.01
session number	0.24	0.98	0.01
age	0.17	0.68	0.01
sex	0.16	0.70	0.01
years of education	0.14	0.71	0.01
protocol * session number	0.13	0.71	0.00
APOE-ε4 status * session number	0.01	0.92	0.00
APOE-ε4 status * protocol * session number	0.00	0.95	0.00

Episodic memory – Recognition – RT - Hits

variable	*F*	*p*	η^2
years of education	6.55	0.02	0.22
age	1.03	0.32	0.04
session number	0.95	0.48	0.03
sex	0.68	0.42	0.03
hours of sleep at Baseline Night	0.62	0.44	0.03
APOE-ε4 status * protocol * session number	2.13	0.15	0.01
APOE-ε4 status * protocol	0.51	0.48	0.01
APOE-ε4 status * session number	1.80	0.18	0.01
protocol * session number	1.78	0.18	0.01
APOE-ε4 status	0.32	0.57	0.01
protocol	0.11	0.75	0.00

Episodic memory – Recognition – RT – Correct Rejections

variable	*F*	*p*	η^2
age	1.12	0.30	0.05
protocol	1.16	0.29	0.03
hours of sleep at Baseline Night	0.65	0.43	0.03
session number	0.80	0.60	0.03
years of education	0.38	0.54	0.02
APOE-ε4 status * protocol * session number	2.36	0.13	0.01
APOE-ε4 status * session number	2.32	0.13	0.01
APOE-ε4 status * protocol	0.31	0.58	0.01
APOE-ε4 status	0.15	0.70	0.00
sex	0.08	0.78	0.00
protocol * session number	0.32	0.57	0.00

Episodic memory – Source Memory – Count - Hits

variable	*F*	*p*	η^2
years of education	4.43	0.05	0.16
sex	2.40	0.13	0.09
session number	0.41	0.92	0.01
hours of sleep at Baseline Night	0.18	0.67	0.01
age	0.08	0.78	0.00
APOE-ε4 status * session number	0.72	0.40	0.00
APOE-ε4 status * protocol * session number	0.30	0.59	0.00
APOE-ε4 status	0.08	0.77	0.00
protocol	0.07	0.79	0.00
protocol * session number	0.01	0.91	0.00
APOE-ε4 status * protocol	0.00	0.98	0.00

Episodic memory – Source Memory – Count - RT

variable	*F*	*p*	η^2
years of education	2.20	0.15	0.09
APOE-ε4 status * protocol	3.59	0.06	0.07
APOE-ε4 status	3.51	0.07	0.07
protocol	3.23	0.08	0.06
sex	1.04	0.32	0.04
session number	0.69	0.70	0.02
hours of sleep at Baseline Night	0.40	0.53	0.02
APOE-ε4 status * protocol * session number	0.53	0.47	0.00
APOE-ε4 status * session number	0.32	0.57	0.00
protocol * session number	0.16	0.69	0.00
age	0.00	0.95	0.00

Statistics for each model – Allocentric navigation – SHQ – wayfinding (Model 2)

SHQ – Wayfinding – easy levels – distance travelled

variable	*F*	*p*	η^2
age	0.69	0.42	0.04
sex	0.46	0.51	0.02
session number	0.60	0.78	0.02
APOE-ε4 status	0.56	0.46	0.02
APOE-ε4 status * protocol	0.48	0.49	0.01
years of education	0.19	0.67	0.01
protocol	0.32	0.58	0.01
APOE-ε4 status * session number	1.38	0.24	0.01
APOE-ε4 status * protocol * session number	1.36	0.24	0.01
protocol * session number	0.35	0.55	0.00
hours of sleep at Baseline Night	0.01	0.91	0.00

SHQ – Wayfinding – hard levels – distance travelled

variable	*F*	*p*	η^2
session number	0.72	0.67	0.03
protocol	0.22	0.64	0.01
APOE-ε4 status * protocol	0.16	0.69	0.01
age	0.08	0.79	0.00
hours of sleep at Baseline Night	0.06	0.81	0.00
APOE-ε4 status * protocol * session number	0.53	0.47	0.00
protocol * session number	0.52	0.47	0.00
APOE-ε4 status	0.06	0.81	0.00
sex	0.03	0.86	0.00
APOE-ε4 status * session number	0.09	0.76	0.00
years of education	0.01	0.93	0.00

SHQ – Wayfinding – easy levels – time to complete

variable	*F*	*p*	η^2
age	1.07	0.31	0.05
APOE-ε4 status * protocol	1.02	0.32	0.03
APOE-ε4 status	0.98	0.33	0.03
years of education	0.50	0.49	0.03
protocol	0.77	0.39	0.02
session number	0.57	0.80	0.02
sex	0.41	0.53	0.02
APOE-ε4 status * session number	2.93	0.09	0.01
APOE-ε4 status * protocol * session number	2.64	0.11	0.01
hours of sleep at Baseline Night	0.12	0.73	0.01
protocol * session number	0.78	0.38	0.00

SHQ – Wayfinding – hard levels – time to complete

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	0.64	0.44	0.03
APOE-ε4 status	0.96	0.34	0.03
session number	0.62	0.76	0.02
protocol	0.17	0.68	0.01
sex	0.10	0.75	0.01
APOE-ε4 status * protocol	0.16	0.70	0.01
age	0.04	0.85	0.00
years of education	0.02	0.88	0.00
APOE-ε4 status * session number	0.06	0.80	0.00
protocol * session number	0.05	0.82	0.00
APOE-ε4 status * protocol * session number	0.05	0.83	0.00

SHQ – Wayfinding – easy levels – mental effort

variable	*F*	*p*	η^2
session number	0.78	0.62	0.03
sex	0.52	0.48	0.02
hours of sleep at Baseline Night	0.49	0.49	0.02
age	0.43	0.52	0.02
protocol	0.31	0.58	0.01
APOE-ε4 status * protocol	0.14	0.71	0.00
APOE-ε4 status * protocol * session number	0.56	0.45	0.00
APOE-ε4 status * session number	0.52	0.47	0.00
years of education	0.04	0.83	0.00
protocol * session number	0.46	0.50	0.00
APOE-ε4 status	0.05	0.83	0.00

SHQ – Wayfinding – hard levels – mental effort

variable	*F*	*p*	η^2
protocol	1.83	0.19	0.07
session number	1.36	0.22	0.05
APOE-ε4 status * protocol	0.92	0.35	0.04
hours of sleep at Baseline Night	0.61	0.44	0.03
protocol * session number	5.16	0.02	0.02
APOE-ε4 status * protocol * session number	2.97	0.09	0.01
APOE-ε4 status	0.23	0.63	0.01
APOE-ε4 status * session number	1.57	0.21	0.01
sex	0.14	0.71	0.01
years of education	0.11	0.74	0.01
age	0.00	0.95	0.00

Statistics for each model – Egocentric navigation – SHQ – flare levels (Model 2)

SHQ – Flare – easy levels – accuracy

variable	*F*	*p*	η^2
age	2.60	0.12	0.11
session number	1.50	0.16	0.05
protocol	0.68	0.42	0.02
APOE-ε4 status * protocol	0.30	0.59	0.01
hours of sleep at Baseline Night	0.20	0.66	0.01
APOE-ε4 status * session number	0.36	0.55	0.00
APOE-ε4 status * protocol * session number	0.16	0.69	0.00
APOE-ε4 status	0.01	0.90	0.00
sex	0.00	0.97	0.00
years of education	0.00	0.99	0.00
protocol * session number	0.00	0.98	0.00

SHQ – flare – hard levels - accuracy

variable	*F*	*p*	η^2
age	3.03	0.10	0.13
APOE-ε4 status * protocol	3.55	0.07	0.08
protocol	2.90	0.10	0.07
APOE-ε4 status	2.56	0.12	0.06
session number	0.49	0.86	0.02
years of education	0.28	0.60	0.01
hours of sleep at Baseline Night	0.17	0.68	0.01
APOE-ε4 status * protocol * session number	0.92	0.34	0.00
sex	0.06	0.81	0.00
APOE-ε4 status * session number	0.51	0.48	0.00
protocol * session number	0.02	0.88	0.00

SHQ – flare – easy levels – time to complete

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	0.91	0.35	0.04
protocol * session number	5.13	0.02	0.02
session number	0.54	0.83	0.02
APOE-ε4 status * protocol * session number	3.18	0.08	0.01
APOE-ε4 status * session number	2.36	0.13	0.01
APOE-ε4 status * protocol	0.32	0.57	0.01
APOE-ε4 status	0.26	0.62	0.01
sex	0.16	0.70	0.01
protocol	0.16	0.69	0.01
age	0.10	0.75	0.00
years of education	0.03	0.87	0.00

SHQ – flare – hard levels – time to complete

variable	*F*	*p*	η^2
age	3.63	0.07	0.15
session number	2.19	0.03	0.07
hours of sleep at Baseline Night	0.78	0.39	0.04
protocol * session number	1.44	0.23	0.01
protocol	0.87	0.35	0.01
sex	0.03	0.86	0.00
APOE-ε4 status * session number	0.05	0.82	0.00
years of education	0.00	0.95	0.00
APOE-ε4 status * protocol * session number	0.04	0.84	0.00
APOE-ε4 status * protocol	0.02	0.90	0.00
APOE-ε4 status	0.01	0.92	0.00

SHQ – Wayfinding – easy levels – mental effort

variable	*F*	*p*	η^2
session number	2.14	0.16	0.09
sex	1.54	0.14	0.05
hours of sleep at Baseline Night	1.23	0.28	0.05
age	1.12	0.30	0.04
protocol	0.52	0.48	0.02
APOE-ε4 status * protocol	0.33	0.57	0.01
APOE-ε4 status * protocol * session number	0.31	0.58	0.01
APOE-ε4 status * session number	0.20	0.66	0.01
years of education	0.44	0.51	0.00
protocol * session number	0.33	0.57	0.00
APOE-ε4 status	0.03	0.86	0.00

SHQ – Flare – hard levels – mental effort

variable	*F*	*p*	η^2
years of education	1.31	0.27	0.09
session number	1.99	0.05	0.05
APOE-ε4 status	3.18	0.09	0.05
APOE-ε4 status * protocol	2.81	0.11	0.04
protocol	2.10	0.16	0.02
age	0.47	0.50	0.01
sex	0.16	0.69	0.01
hours of sleep at Baseline Night	0.12	0.73	0.01
protocol * session number	3.13	0.08	0.00
APOE-ε4 status * protocol * session number	2.92	0.09	0.00
APOE-ε4 status * session number	2.82	0.09	0.00

Appendix 16- *Statistics for Model 3*

Statistics for each model – sleep architecture – Naps

General sleep

Total Time in Bed (TIB)

variable	*F*	*p*	η^2
nap number	3.32	0.002	0.20
sex	9.09	0.003	0.08
hours of sleep at Baseline Night	2.26	0.14	0.02
APOE-ε4 status * nap number	0.68	0.41	0.01
age	0.64	0.43	0.01
APOE-ε4 status	0.02	0.88	0.00

Total Sleep Time (TST)

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	4.84	0.05	0.29
nap number	4.15	≤0.001	0.25
sex	0.57	0.47	0.05
age	0.50	0.50	0.04
APOE-ε4 status	0.91	0.35	0.02
APOE-ε4 status * nap number	0.57	0.45	0.01

Sleep period time

variable	*F*	*p*	η^2
nap number	3.33	0.002	0.21
hours of sleep at Baseline Night	2.21	0.16	0.16
sex	1.76	0.21	0.14
APOE-ε4 status	0.65	0.43	0.02
APOE-ε4 status * nap number	0.12	0.73	0.00
age	0.00	0.99	0.00

Sleep Efficiency (SE)

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	5.39	0.04	0.32
nap number	3.52	≤0.001	0.22
age	0.56	0.47	0.05
APOE-ε4 status	0.90	0.35	0.02
sex	0.24	0.63	0.02
APOE-ε4 status * nap number	0.42	0.52	0.00

Wake after Sleep Onset (WASO)

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	2.40	0.15	0.17
nap number	2.48	0.02	0.17
age	1.63	0.23	0.13
sex	0.53	0.48	0.05
APOE-ε4 status	0.22	0.64	0.01
APOE-ε4 status * nap number	1.08	0.30	0.01

Sleep onset

variable	*F*	*p*	η^2
nap number	2.64	0.01	0.17
hours of sleep at Baseline Night	1.33	0.27	0.10
sex	0.18	0.68	0.02
APOE-ε4 status	0.62	0.43	0.01
APOE-ε4 status * nap number	0.07	0.79	0.00
age	0.01	0.94	0.00

Latency to persistent sleep

variable	*F*	*p*	η^2
age	11.85	0.01	0.52
nap number	1.46	0.18	0.10
APOE-ε4 status * nap number	2.37	0.13	0.02
APOE-ε4 status	0.81	0.37	0.02
sex	0.21	0.66	0.02
hours of sleep at Baseline Night	0.13	0.73	0.01

Latency to N1

variable	*F*	*p*	η^2
nap number	2.18	0.04	0.15
hours of sleep at Baseline Night	1.17	0.30	0.09
APOE-ε4 status * nap number	2.07	0.15	0.02
age	0.05	0.82	0.00
sex	0.03	0.88	0.00
APOE-ε4 status	0.03	0.87	0.00

Latency to N2

variable	*F*	*p*	η^2
nap number	5.86	<0.001	0.33
hours of sleep at Baseline Night	4.48	0.06	0.32
age	1.26	0.29	0.12
sex	0.08	0.78	0.01
APOE-ε4 status * nap number	0.28	0.60	0.00
APOE-ε4 status	0.00	0.96	0.00

Latency to N3

variable	*F*	*p*	η^2
age	0.90	0.37	0.08
nap number	0.45	0.89	0.04
sex	0.39	0.55	0.03
hours of sleep at Baseline Night	0.14	0.72	0.01
APOE-ε4 status	0.35	0.56	0.01
APOE-ε4 status * nap number	0.00	0.97	0.00

Latency to REM

variable	*F*	*p*	η^2
nap number	4.02	0.002	0.50
sex	0.42	0.53	0.04
age	0.38	0.55	0.03
APOE-ε4 status * nap number	0.42	0.52	0.01
APOE-ε4 status	0.06	0.80	0.00
hours of sleep at Baseline Night	0.00	1.00	0.00

Sleep structure

Wake duration

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	5.31	0.04	0.32
nap number	3.91	≤0.001	0.24
age	0.83	0.38	0.07
APOE-ε4 status	2.44	0.13	0.06
sex	0.34	0.57	0.03
APOE-ε4 status * nap number	1.98	0.16	0.02

N1 duration

variable	*F*	*p*	η^2
nap number	2.57	0.01	0.17
APOE-ε4 status	2.67	0.11	0.10
hours of sleep at Baseline Night	0.20	0.66	0.02
sex	0.17	0.68	0.02
APOE-ε4 status * nap number	0.84	0.36	0.01
age	0.01	0.94	0.00

N2 duration

variable	*F*	*p*	η^2
sex	11.59	0.01	0.51
hours of sleep at Baseline Night	4.95	0.05	0.30
nap number	4.79	≤0.001	0.28
age	0.06	0.81	0.01
APOE-ε4 status * nap number	0.04	0.84	0.00
APOE-ε4 status	0.00	0.98	0.00

N3 duration

variable	*F*	*p*	η^2
sex	24.34	≤0.001	0.18
nap number	1.82	0.08	0.12
age	0.96	0.33	0.01
APOE-ε4 status	0.39	0.53	0.00
APOE-ε4 status * nap number	0.09	0.76	0.00
hours of sleep at Baseline Night	0.03	0.87	0.00

REM duration

variable	*F*	*p*	η^2
nap number	11.68	≤0.001	0.48
hours of sleep at Baseline Night	3.98	0.07	0.26
sex	0.56	0.47	0.05
age	0.31	0.59	0.03
APOE-ε4 status	0.33	0.57	0.01
APOE-ε4 status * nap number	0.02	0.97	0.00

N1 percentage of Total Sleep time (TST)

variable	*F*	*p*	η^2
nap number	4.63	≤0.001	0.27
hours of sleep at Baseline Night	1.63	0.23	0.13
sex	0.36	0.56	0.03
age	0.27	0.62	0.02
APOE-ε4 status * nap number	0.19	0.67	0.00
APOE-ε4 status	0.07	0.79	0.00

N2 percentage of Total Sleep time (TST)

variable	*F*	*p*	η^2
sex	9.20	0.01	0.47
hours of sleep at Baseline Night	1.62	0.23	0.13
nap number	1.69	0.11	0.12
age	0.01	0.92	0.00
APOE-ε4 status	0.03	0.86	0.00
APOE-ε4 status * nap number	0.00	0.95	0.00

N3 percentage of Total Sleep time (TST)

variable	*F*	*p*	η^2
sex	29.50	≤0.001	0.21
nap number	1.62	0.13	0.11
age	1.47	0.23	0.01
hours of sleep at Baseline Night	0.83	0.37	0.01
APOE-ε4 status	0.13	0.72	0.00
APOE-ε4 status * nap number	0.11	0.74	0.00

REM percentage of Total Sleep time (TST)

variable	*F*	*p*	η^2
nap number	7.37	≤0.001	0.37
hours of sleep at Baseline Night	6.12	0.03	0.34
age	0.15	0.71	0.01
APOE-ε4 status	0.19	0.67	0.00
sex	0.00	0.95	0.00
APOE-ε4 status * nap number	0.01	0.92	0.00

Sleep continuity

Number of awakenings

variable	*F*	*p*	η^2
nap number	4.43	≤0.001	0.26
age	2.39	0.15	0.18
sex	0.62	0.45	0.05
APOE-ε4 status * nap number	1.69	0.20	0.02
APOE-ε4 status	0.09	0.76	0.00
hours of sleep at Baseline Night	0.00	1.00	0.00

Number of awakenings from N1 sleep

variable	*F*	*p*	η^2
age	3.02	0.11	0.21
nap number	1.85	0.08	0.13
hours of sleep at Baseline Night	1.31	0.28	0.10
sex	0.66	0.43	0.06
APOE-ε4 status * nap number	1.91	0.17	0.02
APOE-ε4 status	0.35	0.56	0.01

Number of awakenings from N2 sleep

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	9.75	0.01	0.47
nap number	2.94	0.01	0.19
age	1.72	0.22	0.13
sex	1.49	0.25	0.12
APOE-ε4 status	0.13	0.72	0.00
APOE-ε4 status * nap number	0.12	0.73	0.00

Number of awakenings from N3 sleep

variable	*F*	*p*	η^2
nap number	2.15	0.04	0.17
sex	0.77	0.40	0.07
hours of sleep at Baseline Night	0.51	0.49	0.05
APOE-ε4 status	0.85	0.36	0.03
APOE-ε4 status * nap number	1.05	0.31	0.01
age	0.07	0.80	0.01

Number of awakenings from REM sleep

variable	*F*	*p*	η^2
nap number	1.02	0.44	0.19
APOE-ε4 status	1.74	0.20	0.05
sex	0.35	0.57	0.03
APOE-ε4 status * nap number	1.22	0.28	0.03
age	0.21	0.65	0.01
hours of sleep at Baseline Night	0.00	0.95	0.00

Sleep stability

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	4.41	0.06	0.29
nap number	2.21	0.03	0.15
age	1.22	0.29	0.10
sex	0.28	0.61	0.03
APOE-ε4 status * nap number	0.33	0.57	0.00
APOE-ε4 status	0.04	0.84	0.00

Fast sleep changes

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	2.51	0.14	0.19
nap number	1.68	0.11	0.12
sex	0.67	0.43	0.06
age	0.64	0.44	0.06
APOE-ε4 status	0.67	0.42	0.02
APOE-ε4 status * nap number	1.38	0.24	0.01

Deep sleep changes

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	1.45	0.25	0.12
nap number	1.17	0.32	0.09
sex	0.24	0.63	0.02
age	0.04	0.84	0.00
APOE-ε4 status * nap number	0.34	0.56	0.00
APOE-ε4 status	0.07	0.79	0.00

Shallow sleep changes

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	1.39	0.26	0.11
nap number	1.20	0.30	0.09
sex	0.21	0.66	0.02
APOE-ε4 status	0.30	0.59	0.01
APOE-ε4 status * nap number	0.74	0.39	0.01
age	0.00	0.95	0.00

Big Shallow changes

variable	*F*	*p*	η^2
nap number	5.74	<0.001	0.32
age	0.37	0.56	0.04
sex	0.05	0.84	0.00
APOE-ε4 status	0.00	0.99	0.00
hours of sleep at Baseline Night	0.07	0.79	0.01
APOE-ε4 status * nap number	0.03	0.85	0.00

Sleep fragmentation

Sleep fragmentation

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	6.89	0.02	0.38
age	3.66	0.08	0.25
nap number	2.48	0.02	0.17
sex	0.46	0.51	0.04
APOE-ε4 status * nap number	2.23	0.14	0.02
APOE-ε4 status	0.64	0.43	0.02

Entries to N1

variable	*F*	*p*	η^2
nap number	3.62	<0.001	0.23
age	1.73	0.21	0.14
sex	0.30	0.60	0.03
APOE-ε4 status	0.74	0.40	0.03
hours of sleep at Baseline Night	0.15	0.71	0.01
APOE-ε4 status * nap number	0.65	0.42	0.01

N1 Fragmentation

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	0.02	0.90	0.00
sex	0.03	0.87	0.00
APOE-ε4 status * nap number	0.05	0.83	0.00
age	0.21	0.66	0.02
APOE-ε4 status	0.23	0.64	0.01
nap number	1.03	0.42	0.08

Entries to N2

variable	*F*	*p*	η^2
nap number	1.93	0.06	0.14
sex	0.41	0.53	0.04
hours of sleep at Baseline Night	0.24	0.63	0.02
APOE-ε4 status	0.52	0.48	0.02
age	0.17	0.69	0.02
APOE-ε4 status * nap number	0.82	0.37	0.01

N2 Fragmentation

variable	*F*	*p*	η^2
hours of sleep at Baseline Night	11.82	0.01	0.52
nap number	1.96	0.06	0.14
sex	1.17	0.30	0.10
age	0.10	0.75	0.01
APOE-ε4 status	0.10	0.76	0.00
APOE-ε4 status * nap number	0.13	0.72	0.00

Entries to N3

variable	*F*	*p*	η^2
age	3.58	0.09	0.26
nap number	1.39	0.21	0.10
sex	0.23	0.64	0.02
APOE-ε4 status * nap number	0.78	0.38	0.01
hours of sleep at Baseline Night	0.00	0.95	0.00
APOE-ε4 status	0.01	0.91	0.00

N3 Fragmentation

variable	*F*	*p*	η^2
sex	6.71	0.03	0.42
nap number	1.59	0.14	0.13
APOE-ε4 status	2.09	0.16	0.05
APOE-ε4 status * nap number	1.81	0.18	0.02
hours of sleep at Baseline Night	0.20	0.66	0.02
age	0.11	0.75	0.01

Entries to REM

variable	*F*	*p*	η^2
age	0.85	0.38	0.26
nap number	9.49	<0.001	0.10
sex	1.05	0.33	0.02
APOE-ε4 status * nap number	0.55	0.46	0.01
hours of sleep at Baseline Night	5.21	0.04	0.00
APOE-ε4 status	0.06	0.81	0.00

REM Fragmentation

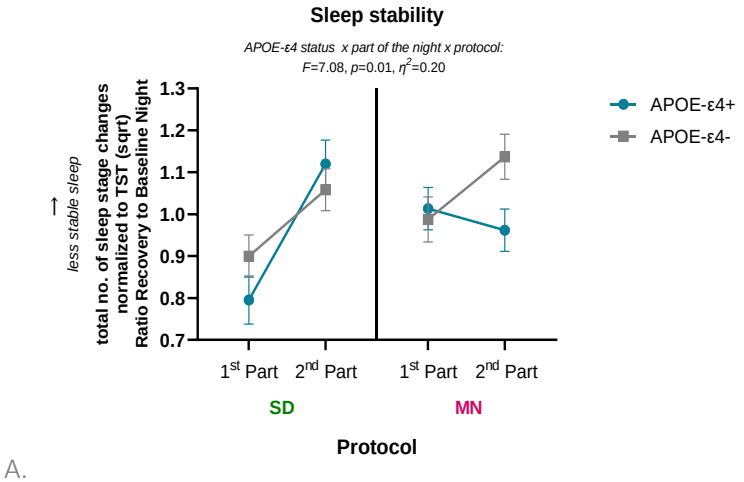
variable	*F*	*p*	η^2
nap number	4.28	0.002	0.58
APOE-ε4 status	4.97	0.03	0.14
APOE-ε4 status * nap number	3.56	0.07	0.11
sex	0.41	0.55	0.07
age	0.28	0.61	0.04
hours of sleep at Baseline Night	0.00	0.97	0.00

Note, the residuals of model involving *Big deep sleep changes* were strongly skewed and failed to be normalized using data transformations, hence the model was not included into the appendix.

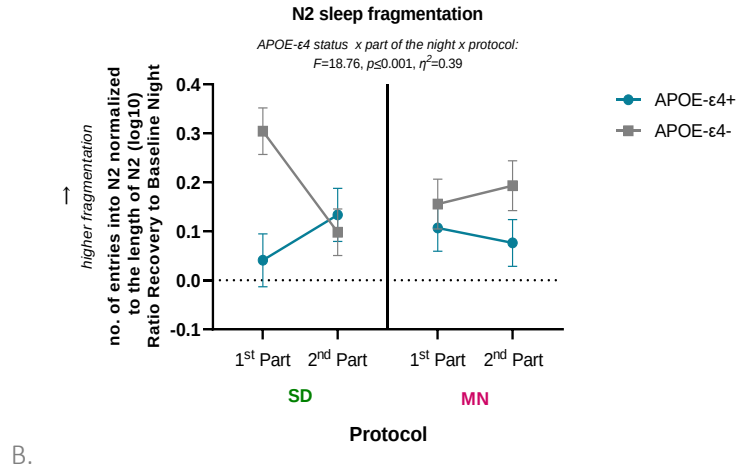
Appendix 17 – *Statistics – Model 4*

To investigate an interaction between APOE-ε4 status, protocol and part of the night (first versus second half) following mixed model was computed: Model 4*:* lmer (*variable of interest* ~ age + sex + APOE-ε4 status + protocol + part of the night + APOE-ε4 status*protocol + APOE-ε4 status*part of the night + protocol*part of the night + APOE-ε4 status*protocol*part of the night + (1|id)). The dependent variables were expressed as ratio between Recovery and Baseline night.

The three-way interaction of interest (APOE-ε4 status*protocol*part of the night) reached significance for sleep stability (p=0.01, η^2=0.20) (Figure 1A), N2 sleep fragmentation ($p \leq 0.001$, η^2=0.39) (Figure 1B) and marginal significance for N1 entries (p=0.06, η^2=0.11), deep sleep changes (p=0.06, η^2=0.06), shallow sleep changes (p=0.07, η^2=0.11), big shallow sleep changes (p=0.09, η^2=0.10), awakenings from N2 (p=0.12, η^2=0.11) and sleep efficiency (p=0.12, η^2=0.08)



A.



B.

Figure 1. Interactions between APOE-ε4 status, protocol and part of the night in the context of the homeostatic response to sleep loss. A. Sleep stability B. N2 fragmentation. The values are expressed as ratio of Baseline to Recovery Night and . Data points are expressed as least-square means values ±SE.

	Dependent variable	*Predictors*																											
		age			*sex*			APOE- $\varepsilon4$ status			*protocol*			part of the night			APOE- $\varepsilon4$ status * protocol			APOE- $\varepsilon4$ status * part of the night			protocol * part of the night			APOE-$\varepsilon4$ status * protocol * part of the night		
		F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
General sleep parameters	Time in bed	3.40	0.08	0.11	0.11	0.74	0.00	1.49	0.23	0.05	4.17	0.05	0.13	0.02	0.89	0.00	1.45	0.24	0.05	0.04	0.84	0.00	0.26	0.61	0.01	0.15	0.70	0.01
	Total sleep time (sqrt)	1.74	0.20	0.06	2.46	0.13	0.08	3.20	0.08	0.08	3.85	0.06	0.09	2.54	0.12	0.08	3.21	0.08	0.08	1.60	0.22	0.05	1.85	0.18	0.06	1.96	0.17	0.06
	Sleep period time	2.58	0.12	0.09	0.29	0.59	0.01	0.05	0.83	0.00	0.37	0.54	0.01	0.69	0.41	0.02	0.01	0.91	0.00	0.62	0.44	0.02	1.36	0.25	0.04	1.06	0.31	0.04
	Sleep efficiency	0.01	0.92	0.00	1.53	0.23	0.05	2.80	0.10	0.07	2.55	0.12	0.06	3.13	0.09	0.10	2.69	0.11	0.06	2.31	0.14	0.07	2.28	0.14	0.07	2.59	0.12	0.08
	Wake after sleep onset (sqrt)	0.09	0.76	0.00	0.93	0.34	0.02	0.01	0.94	0.00	0.48	0.49	0.01	0.47	0.49	0.01	0.16	0.69	0.00	0.01	0.93	0.00	0.75	0.39	0.01	0.18	0.67	0.00
Sleep structure	Wake duration (sqrt)	0.22	0.64	0.01	0.51	0.48	0.02	0.04	0.84	0.00	0.67	0.42	0.02	0.62	0.44	0.02	0.22	0.64	0.01	0.00	1.00	0.00	0.88	0.36	0.03	0.25	0.62	0.01
	N1 duration (sqrt)	0.29	0.60	0.01	0.03	0.85	0.00	0.58	0.45	0.01	1.65	0.21	0.04	2.27	0.14	0.07	0.37	0.54	0.01	0.85	0.36	0.03	2.04	0.16	0.07	0.72	0.40	0.02
	N2 duration (sqrt)	0.01	0.92	0.00	0.23	0.63	0.01	3.00	0.09	0.07	0.60	0.44	0.02	0.37	0.55	0.01	1.70	0.20	0.04	1.68	0.21	0.05	0.29	0.60	0.01	1.17	0.29	0.04
	N3 duration (log10)	2.27	0.14	0.04	0.10	0.75	0.00	0.29	0.59	0.01	0.08	0.78	0.00	0.01	0.91	0.00	0.37	0.54	0.01	0.06	0.80	0.00	0.02	0.88	0.00	0.11	0.74	0.00
	REM duration (log10)	2.79	0.10	0.05	0.87	0.35	0.02	0.35	0.56	0.01	0.12	0.73	0.00	0.21	0.65	0.00	1.31	0.26	0.02	0.39	0.53	0.01	0.31	0.58	0.01	1.21	0.28	0.02
	N1, % of TST (sqrt)	0.04	0.85	0.00	0.05	0.82	0.00	0.74	0.39	0.02	2.19	0.15	0.05	2.50	0.12	0.08	0.61	0.44	0.01	0.77	0.39	0.03	2.17	0.15	0.07	0.83	0.37	0.03
	N2, % of TST	0.27	0.60	0.01	0.34	0.57	0.01	1.16	0.29	0.03	0.03	0.86	0.00	0.04	0.84	0.00	0.33	0.57	0.01	0.68	0.42	0.02	0.01	0.91	0.00	0.27	0.61	0.01
	N3, % of TST (log10)	3.25	0.08	0.06	0.49	0.49	0.01	0.76	0.39	0.01	0.41	0.52	0.01	0.16	0.69	0.00	0.90	0.35	0.02	0.24	0.63	0.00	0.15	0.70	0.00	0.36	0.55	0.01
	REM, % of TST (log10)	1.48	0.23	0.03	0.27	0.61	0.00	0.08	0.77	0.00	0.00	0.97	0.00	0.07	0.80	0.00	0.70	0.41	0.01	0.24	0.62	0.00	0.15	0.70	0.00	0.84	0.36	0.02
Sleep continuity	Number of awakenings (sqrt)	0.52	0.48	0.02	0.42	0.52	0.02	0.99	0.33	0.02	0.02	0.90	0.00	0.90	0.35	0.03	0.84	0.37	0.02	0.02	0.88	0.00	0.89	0.35	0.03	0.00	1.00	0.00
	Awakenings from N1 (sqrt)	0.01	0.93	0.00	0.59	0.45	0.03	0.30	0.59	0.01	2.00	0.17	0.06	4.32	0.05	0.14	0.32	0.58	0.01	0.92	0.35	0.03	3.58	0.07	0.13	0.92	0.35	0.04
	Awakenings from N2 (sqrt)	2.66	0.12	0.11	0.35	0.56	0.02	3.21	0.08	0.08	3.36	0.07	0.08	0.92	0.35	0.04	4.37	0.04	0.11	2.11	0.16	0.08	1.88	0.18	0.08	2.65	0.12	0.11
	Awakenings from N3 (sqrt)	1.08	0.31	0.03	1.39	0.25	0.04	2.05	0.16	0.06	1.05	0.31	0.03	0.22	0.64	0.01	1.34	0.26	0.04	0.23	0.63	0.01	0.01	0.92	0.00	0.06	0.81	0.00
	Awakenings from REM (sqrt)	1.61	0.21	0.04	0.99	0.33	0.02	1.80	0.19	0.04	1.59	0.21	0.04	0.40	0.53	0.01	1.38	0.25	0.03	0.76	0.39	0.02	0.51	0.48	0.01	0.49	0.49	0.01
	Sleep stability (sqrt)	0.01	0.92	0.00	1.13	0.30	0.04	4.65	0.04	0.11	9.39	0.004	0.20	11.89	0.002	0.29	4.97	0.03	0.12	5.85	0.02	0.17	11.37	0.002	0.28	7.08	0.01	0.20
	Fast sleep stage changes (sqrt)	1.05	0.31	0.04	0.18	0.67	0.01	0.16	0.69	0.00	0.32	0.58	0.01	0.02	0.89	0.00	0.67	0.42	0.02	0.01	0.94	0.00	0.01	0.92	0.00	0.15	0.71	0.00

	Deep sleep stage changes (log10)	0.93	0.34	0.02	1.22	0.27	0.02	3.25	0.08	0.05	6.23	0.02	0.10	7.07	0.01	0.11	3.28	0.08	0.06	3.57	0.06	0.06	6.06	0.02	0.10	3.80	0.06	0.06
	Shallow sleep stage changes (sqrt)	0.49	0.49	0.02	0.65	0.43	0.02	2.77	0.10	0.07	5.30	0.03	0.12	5.61	0.02	0.16	2.91	0.10	0.07	2.96	0.10	0.09	5.05	0.03	0.15	3.53	0.07	0.11
	Big deep sleep stage changes (sqrt)	1.36	0.26	0.08	0.20	0.66	0.01	0.51	0.49	0.04	0.26	0.62	0.02	0.46	0.52	0.05	0.39	0.54	0.03	0.72	0.42	0.07	0.39	0.55	0.04	0.39	0.55	0.04
	Big shallow sleep stage changes (log10)	0.26	0.62	0.01	0.01	0.90	0.00	1.18	0.28	0.03	0.59	0.45	0.02	1.56	0.22	0.05	1.33	0.26	0.03	3.14	0.09	0.10	1.93	0.17	0.06	3.08	0.09	0.10
Sleep fragmentation	Sleep fragmentation (sqrt)	0.15	0.71	0.01	0.03	0.86	0.00	0.38	0.54	0.01	0.07	0.79	0.00	1.15	0.29	0.04	0.22	0.64	0.01	0.00	0.98	0.00	1.17	0.29	0.04	0.07	0.80	0.00
	N1 entries (sqrt)	0.25	0.62	0.01	0.04	0.84	0.00	2.18	0.15	0.05	4.80	0.03	0.10	7.57	0.01	0.21	2.14	0.15	0.05	3.53	0.07	0.11	7.40	0.01	0.20	3.73	0.06	0.11
	N1 fragmentation (log10)	0.02	0.88	0.00	0.06	0.80	0.00	0.22	0.64	0.01	0.13	0.72	0.00	0.62	0.44	0.02	0.25	0.62	0.01	0.49	0.49	0.02	0.61	0.44	0.02	0.54	0.47	0.02
	N2 entries (log10)	0.00	0.99	0.00	1.69	0.20	0.06	0.73	0.40	0.02	1.29	0.26	0.03	2.37	0.13	0.08	0.64	0.43	0.02	1.39	0.25	0.05	1.95	0.17	0.06	1.20	0.28	0.04
	N2 fragmentation (log10)	0.11	0.75	0.00	0.86	0.36	0.03	20.59	≤0.001	0.28	9.33	0.004	0.15	16.08	≤0.001	0.36	13.53	≤0.001	0.20	24.16	≤0.001	0.45	13.10	≤0.001	0.31	18.76	≤0.001	0.39
	N3 entries (sqrt)	0.61	0.44	0.02	0.01	0.93	0.00	1.15	0.29	0.03	1.44	0.24	0.03	0.64	0.43	0.02	1.07	0.31	0.03	0.57	0.46	0.02	0.43	0.52	0.01	0.50	0.48	0.02
	N3 fragmentation (log10)	1.02	0.32	0.02	0.24	0.62	0.00	0.07	0.80	0.00	0.46	0.50	0.01	0.28	0.60	0.01	0.02	0.89	0.00	0.10	0.75	0.00	0.13	0.72	0.00	0.03	0.86	0.00
	REM entries (sqrt)	0.47	0.50	0.02	3.91	0.06	0.13	0.14	0.71	0.00	0.07	0.80	0.00	0.00	0.97	0.00	0.29	0.59	0.01	0.01	0.92	0.00	0.03	0.86	0.00	0.00	0.99	0.00
	REM fragmentation (log10)	0.43	0.52	0.02	1.73	0.20	0.06	0.03	0.86	0.00	0.01	0.94	0.00	0.31	0.58	0.01	0.29	0.59	0.01	0.67	0.42	0.02	0.72	0.40	0.02	1.40	0.25	0.05

Table 1. Outcomes of mixed model including the three-way interaction term between APOE-ε4 carrier and part of the night and protocol. Statistical test and measure of effect size: Mixed-effects model & eta squared. The dependent variable is a ratio between Recovery to Baseline Night. Used transformation (dependent variable): log10 – log10-transformation, ^2 – power transformation, sqrt – square root transformation. For a definition of the outcome measures please refer to Table 12 in the Methods section.